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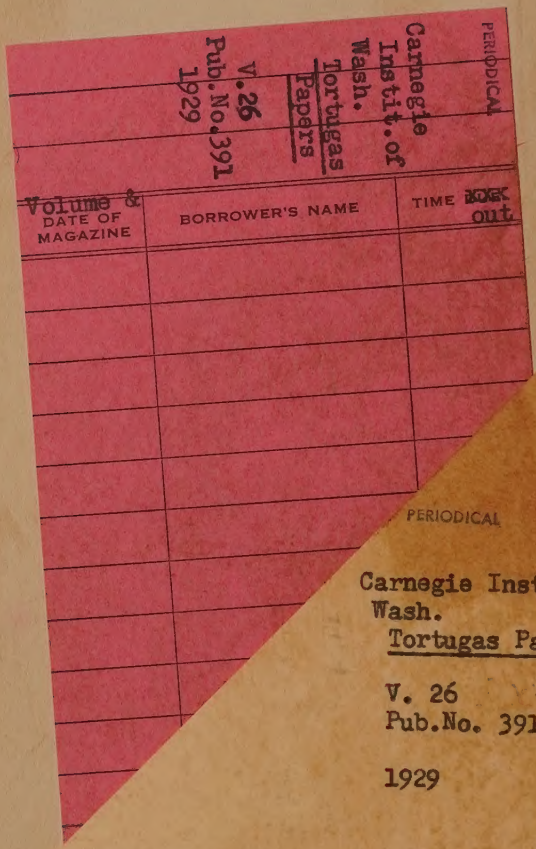

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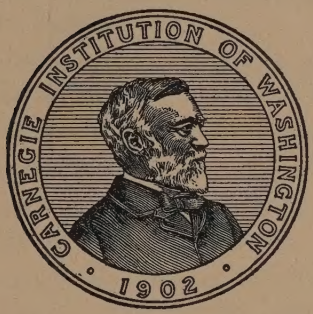

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PAPERS FROM THE TORTUGAS LABORATORY

OF

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VOLUME XXVI

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II. Activation of the Eggs of Echinometra mathæi by Sperms of the Crinoids Comatula pectinata and Comatula purpurea. By David H. Tennent.

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I

AN INVESTIGATION ON ORGANIZATION IN A SEA-URCHIN EGG

By D. H. Tennent, C. V. Taylor and D. M. Whitaker

Fifty-nine text-figures

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AN INVESTIGATION ON ORGANIZATION IN A SEA-URCHIN EGG

INTRODUCTION

The fact that sea-urchin eggs may be fragmented by shaking, and that the fragments, both nucleated and non-nucleated, may be fertilized, was discovered by O. and R. Hertwig ('87). This method of obtaining egg fragments was used by Boveri ('89, '95, etc.), Morgan ('94, '95), Driesch ('93, etc.) and other investigators in various studies involving the use of fertilized nucleated and non-nucleated fragments. Boveri also used experimentally a mechanical device of the nature of a small guillotine for cutting a fragment from the egg, but found that the fragments which he obtained by this procedure were less satisfactory than those obtained by shaking.

Delage ('98, '99) criticised the method of fragmentation by shaking, on the ground of its violence, but offered no explanation of the nature of the harmful effect. He found it possible to cut eggs singly, by hand, under the microscope, and observed that both the nucleated and non-nucleated fragments were capable of fertilization and development. His statement that "the segmentation takes place exactly as in the normal egg," is not confirmed either by text or illustrations. There is no evidence that he observed such details as the formation of micromeres. It may be said, however, that Delage, by an exact method, established the fact of "merogonie," which was his designation for the development of a non-nucleated egg fragment following its fertilization by a spermatozoön. Verworn ('92), in a critical consideration of the significance of the cell nucleus, in which he reviewed Boveri's work, regarded the phenomenon as a sort of male parthenogenesis, "androgenesis," while Rawitz ('01) gave it the name "ephebogenesis."

The results obtained by Boveri during the investigations that formed the basis of his paper *Zwei Fehlerquellen bei Merogonieversuchen und die Entwicklungsfähigkeit merogonischer und partiell merogonischer Seeigelbastarde,* published in 1918, a year after his death, showed that conclusions based on mass fragmentation of sea-urchin eggs were untrustworthy. His criticism was not of the fact of merogony, although there was at the time a rather widespread conviction that this was its purport, but a criticism of a method. Boveri showed that, during the process of shaking, the nucleus may collapse and become invisible. The fact that no nucleus is visible in a frag-

[1] Most of the illustrations were drawn by Miss Margaret Hill Collins under the direction of one of the authors.

ment is not a proof that the fragment is non-nucleated. A stained preparation may show the presence of a group of chromosomes. His studies showed that in fragments that had been selected as non-nucleated and then fertilized, the nucleus might have the diploid rather than the haploid size. Boveri also suggested that when unfertilized eggs containing scattered partial nuclei are shaken, their fragments may contain invisible partial nuclei. Fragments of this kind, if they developed after fertilization, might contain some of the maternal chromosomes. Boveri's evidence is convincing in its demonstration that the nature of fragments obtained by shaking the egg to pieces is unknown, so far as nuclear content is concerned.

As a result of a discussion of Boveri's paper, between two of the authors of this report, it was decided to undertake in collaboration an investigation of merogonic sea-urchin hybrids with the aid of improved methods of micromanipulation. These studies were begun early in January 1923, at the Hopkins Marine Station, Pacific Grove, California, and were based on *franciscanus* and *purpuratus*, two species of *Strongylocentrotus*. Preliminary work was done on the effects on development produced by flattening, puncturing and cutting the egg, as well as on the development of egg fragments fertilized by sperms of their own species. When this was completed no time remained for the studies that had been planned. Some of the results were so encouraging that it was agreed to continue the collaboration as soon as possible. An opportunity to do this came in 1924 through the invitation of the Carnegie Institution of Washington to work at the Tortugas Laboratory.

The eggs of the sea-urchins *Lytechinus (Toxopneustes) variegatus* and *Tripneustes (Hipponoë) esculenta*, which may be obtained in abundance at Tortugas, were very familiar to one of us, and a quick solution of our problem was anticipated. It soon became evident that an exact knowledge was necessary of the comparative development of species fertilized animal and vegetal halves of the egg, and of halves obtained by section through the poles.

It was assumed that the *Lytechinus* egg had before fertilization the same type of organization as that described by Boveri ('01) for *Paracentrotus*. It seemed probable that from an animal half we should get a larva in which there would be neither mesenchyme nor archenteron, and from a vegetal half a normal gastrula and pluteus. It was supposed further, that, from the halves obtained by cutting the egg through the poles, identical larvæ would be secured if bilaterality were not established before the spermatozoön entered the egg.

Contrary to these expectations, the results showed that *both halves of the egg, no matter what the plane of section, might form normal identical larvæ*. The animal halves, like the vegetal halves, produced larvæ with mesenchyme and an archenteron. These larvæ were, furthermore, similar in every respect to those from halves resulting from section through the poles, or in any other plane.

These results indicated that potential micromere-forming material and endoderm were not localized in the *Lytechinus* egg before fertilization, and it seemed desirable to make a study of the cleavage of egg fragments obtained by cutting eggs in various planes, in order to ascertain the ability of a given fragment to form micromeres.

With the equipment at our command the observational work went slowly because of the difficulty in making observations quickly on both fragments of an egg. It was found impossible to finish the preliminary investigation on organization before an unfavorable condition of the *Lytechinus* material for experimental work made it necessary to suspend our studies.

Further collaborative work was delayed for two years. In June 1926, Mr. Douglas M. Whitaker joined us in the continuation of these studies at the Tortugas Laboratory. During the ensuing eight weeks our previous record on the development of species-fertilized fragments was checked and extended by the addition of new data.

The facts presented concerning the early development of pairs of fragments obtained by cutting the eggs of *Lytechinus variegatus* have been established by a carefully controlled investigation. Nothing has been left to surmise or conjecture. Nothing is inferred concerning any other egg.

Two preliminary papers have been published, one in the Carnegie Institution Year Book No. 23, after the close of the work in 1924, the other after the completion of the work in 1926, in the Carnegie Institution Year Book No. 25.

MATERIAL AND METHODS

The eggs used in our experiments at Tortugas were those of *Lytechinus variegatus*. These eggs are about 0.1 to 0.12 mm. in diameter, sometimes slightly elongated in the direction of the polar axis. They are transparent enough to permit the nucleus to be seen readily. They were obtainable in abundance and were remarkably well suited to the needs of the investigation. The nucleus of the *Lytechinus* egg, like that of *Paracentrotus (Strongylocentrotus)*, which was used by Boveri, lies indifferently in the animal or vegetal half of the egg, and with respect to the primary axis is often eccentric in position. Its variable position possibly depends on the degree of viscosity of the cytoplasm in which it lies. We were inclined to believe that it lies more often in the vegetative half of the egg than in the animal half. A test, however, of this impression, based on the examination of 156 of the eggs that were cut, showed that in 75 eggs the nucleus was in the animal half, in 58 in the vegetative half, and in 23 in the center of the egg.

The sea-urchins from which our material was obtained were collected each morning and brought to the laboratory as quickly as possible. Work on the material was begun immediately upon its arrival. Throughout the

work the greatest possible care in the handling of material, instruments and glassware was taken in order to avoid contamination of the eggs.

In 1924 we adhered rigidly to the following method of procedure. Eggs which were extruded through the gonopores of a horizontally transected female, or eggs which exuded freely from the cut surface of a thoroughly ripe ovary were pipetted carefully to a glass dish containing about 600 c.c. of fresh sea-water. They were allowed to settle and were then washed with one change of sea-water. In from 5 to 10 minutes this stock began to serve as a supply for the work of from 1 to 2 hours, when a new stock from freshly opened females was prepared. The number of eggs in a given culture was never allowed to exceed a few hundred and formed a scattered layer on the bottom of the dish. From the stock a single egg was transferred, as wanted, to a small drop of sea-water on a scrupulously clean cover-slip. If ink were used for the demonstration of the funnel, or "micropyle," in the jelly layer, a minute droplet of a suspension of fresh octopus ink in sea-water was added at this time. Water, or water and ink, was then drawn, with a lip pipette, from the drop until its surface film compressed the egg slightly. The cover-slip was then inverted over the moist chamber on the stage of a compound microscope and the egg brought into focus just above the tip of the micro-needle. When both the egg and tip of the needle were in focus under the 16 mm. objective, the tip of the needle was gradually raised into the drop of water containing the egg. If necessary the egg was carefully turned with the side of the needle-tip so that it could be transected in any desired plane.

The orientation of the eggs before cutting, made possible by the location of the micropyle and the polar bodies, was verified by a second observer. The oriented egg was cut under a magnification of 200 diameters (ocular 5X objective 16 mm.). The magnification was sufficient to enable the operator to know whether the egg had slipped or turned during the operation.

The use of octopus ink was not from choice, but of necessity. It was desired to use Boveri's method for demonstrating the micropyle in order to distinguish between animal and vegetal poles and to ascertain the relation of the position of the cleavage planes and the micromeres to the micropyle. There was no Chinese ink available and attempts at a successful use of vital stains failed. It was found that fresh ink taken from the ink sac of octopus was a satisfactory substitute. The use of Chinese ink is more convenient than the fresh octopus ink, as the latter can not always be had when needed and a reserve supply soon becomes unfit for use because of putrefactive changes by which substances are formed which seem to inhibit the development of the egg.

After the egg had been cut the nucleated and non-nucleated fragments were transferred by means of a lip pipette, each to an embryological watch-glass, "salt cellar," containing 8 c.c. of fresh sea-water. When the frag-

ments had rounded up well (from 5 to 20 minutes after cutting), 2 or 3 drops of a light sperm suspension were added to each dish. Shortly after the appearance of the fertilization membrane, each fragment was transferred to another watch-glass of fresh sea-water. Thus isolated, each developing fragment was followed carefully throughout its succeeding stages. On a few occasions a pair of fragments was kept in the same watch-glass in order that both might be kept under observation continuously. The sea-water in the watch-glass was changed usually once, sometimes twice, daily. When not under observation the watch-glasses were either covered or kept in moist chambers to prevent concentration of the sea-water by evaporation. By this method many fragments, both nucleated and non-nucleated, were reared to a reasonably normal pluteus stage. We first attempted the study of the fragments in a hanging drop in a shallow moist chamber placed on the stage of the microscope. Fragments isolated in a drop of water in this manner usually failed in their development before the second cleavage.

When the cutting was done slowly, with a needle that was not too sharp, in such a manner that the surface layers of the egg were brought together before the actual severing of connection between the two parts, the fragments rounded up well and upon insemination formed well-separated membranes. On the other hand, when the cutting was done quickly, with an exceedingly sharp needle, the fragments remained nearly flat on the surface of section and the fertilization membrane formed only on the more convex surface. The needles used during 1924 were drawn from Pyrex glass. Quartz needles were used during 1926.

The weakest part of this procedure lies practically in the impossibility of making closely consecutive observations on several pairs of fragments. The unavoidable agitation of the water in the watch-glasses when they were moved from the stage of the microscope to the table, or vice versa, usually moved the fragments, and several valuable minutes were lost before they could be located. When the work in 1924 was suspended it was with the conviction that the method of observation must be improved.

In 1926 the observational work was facilitated by a device which made it possible to keep the eggs under practically continuous observation. A metal bar 75 cm. long, in cross-section of the form of a truncate wedge, was fastened to the top of the table. A compound microscope was clamped to a rigid base which slid freely on the horizontally placed metal bar. A glass shelf, of the width of the stage of the microscope, supported at each end, was held so that it just cleared the stage of the microscope. The watch-glasses containing the fragments for study were placed on the glass shelf and the fragments centered under the objective. It was therefore possible to avoid the loss of time that must occur if the watch-glasses have to be picked up and placed on the stage of the microscope for every observation. As the microscope moved freely from side to side on the horizontal bar, the

fragments in from 15 to 20 watch-glasses could be examined repeatedly in a very few minutes.

During the latter part of our work it was found that the micropyle may be seen more readily if the eggs are dropped into the suspension of ink immediately after extrusion from the gonopores, without being washed. Clean eggs must be used, eggs that have not come into contact with cœlomic liquid or materials from the digestive tract. Several eggs were taken in a drop from the suspension, placed on a coverslip, and the size of the drop reduced by the withdrawal of water. The coverslip was then inverted over the moist chamber and a selected egg cut in the desired plane.

The eggs were cut with little, in some instances with no compression. The two fragments, one nucleated and the other non-nucleated, were picked up with a lip pipette and transferred, after being washed three times, to a watch-glass of 1 c.c. capacity. (These watch-glasses were of the Syracuse watch-glass type, about 2.5 cm. in diameter and are known as the Bureau of Plant Industry Model.)

The watch-glass was then placed on the glass shelf and the egg fragments found and sketched. They were then inseminated by dropping one drop of light sperm suspension immediately above their position in the watch-glass. The sperm suspension was prepared by adding one drop of freshly extruded dry sperm to 15 c.c. of sea-water, mixing thoroughly, and diluting further by mixing 1 c.c. of this first sperm suspension with 15 c.c. of sea-water. As has been noted, one drop of this second, or light, suspension was used in inseminating each pair of egg fragments.

After noting whether the fertilization membrane was formed, the dishes were covered by an inverted dish of the same type and kept covered, except during observation, in order to prevent evaporation. The rate and nature of cleavage through the 8-cell stage could be followed readily under low magnification (Zeiss 4 AA).

A special method of observation was necessary for the succeeding divisions. It is one thing to observe the formation of micromeres in a mass fertilization, where if the observation can not be made on one egg it can be made on another. It is quite another thing to make a thoroughly satisfactory observation on two isolated fragments.

The dividing fragments were observed under low magnification until they entered the 8-cell stage. Then, with the aid of a binocular microscope and a lip pipette, they were transferred to a slide and covered with a coverslip supported by glass rods. Under the binocular the position of the dividing fragments was noted and marked by touching the surface of the cover near them with the wet tip of the pipette. The further development of the fragments was then observed under a magnification of 400 diameters (4 D).

For a time, until a high degree of certainty in making the observations had been developed, it was found of advantage to stain the fragments lightly

with neutral red. Enough Grübler's Neutral Red (Zu Inject. i. vital Gew. nach Ehrlich) to tinge the water slightly was rubbed up in 1 c.c. of sea-water. One drop of this solution was added to the dish containing the fragments before they were transferred. The process of transfer took only a minute or two. When the transfer had been made and the examination begun, the dye had been taken up by the "neutral red granules" in the egg. The cell boundaries stood out more clearly in the stained than in the unstained eggs. Although the use of neutral red, in the dilute solution that we employed, did not seem to be in any way harmful to development, its use was discontinued as soon as facility in making the observations had been acquired.

Under the higher magnification thoroughly satisfactory observations were possible. The cleavage amphiasters could be seen clearly as they formed. When necessary the fragments were rotated into the position most favorable for observation by slight manipulation of the coverslip. The cleavages were followed as they were occurring.

Any uncertainty as to the number and origin of the micromeres, and of any other cells resulting from the fourth and fifth or immediately subsequent cleavages was avoided by the study of the fragments continuously under adequate magnification. After the determinations concerning micromeres were made, the cleaving fragments, when further information was desired, were removed from the slides and transferred to watch-glasses for rearing.

PRELIMINARY CONSIDERATION OF THE PLANES OF SECTION

Boveri's ('01) observations on *Paracentrotus* eggs were so conclusive that they seemed to constitute a demonstration of the nature of organization prior to fertilization in that egg. In eggs which he obtained at Villefranche, he found that scattered pigment became concentrated, during maturation, in a ring-like zone lying in the vegetative half of the egg and standing at right angles to the egg axis. He concluded that "the three zones made evident in the ripe egg by the pigment ring correspond to the three primitive organs of the larva; the vegetative, unpigmented cap furnishes the primary mesenchyme and likewise therefore the larval skeleton, the pigmented zone forms the gut and its derivatives, the unpigmented animal half of the egg provides the ectoderm and its differentiations."

Boveri's results led to the generally accepted inference that a similar zone exists in the eggs of other sea-urchins, although it is not made visible by the localization of pigment, and that this zone separates the animal half, containing potential ectoderm, from a vegetative cap of micromere-forming material.

When we began our work we assumed that prior to fertilization in the *Lytechinus* egg there was a complete localization of primary mesenchyme-forming material at the vegetative pole, and that this material was segre-

gated in the micromeres formed at the fourth cleavage. This assumption was in complete agreement with the accepted inference that had been drawn from Boveri's work.

Proceeding with the investigation, we verified the fact that the first two cleavage planes passed through the poles, and that the micromeres were formed at the intersection of these two planes at the vegetal pole. The definitive polarity of the egg, usually regarded as established at the time of maturation, corresponds with the antero-posterior differentiation of the larva. The primary mesenchyme, derived from the micromeres, is proliferated from the posterior wall of the blastula from the region in which the micromeres sink into the wall during the early cleavage of the egg.

Wilson (Wilson and Mathews, '95) showed that the plane of the first cleavage in *Lytechinus* was fixed by three points, the two poles and the point of entrance of the spermatozoön. As the future anterior and posterior ends of the larva are determined before fertilization, if symmetry is established by the plane of the first cleavage, this plane may be either a median dorso-ventral plane, the plane of bilateral symmetry, or it may be horizontal, a mid-frontal plane. If bilateral symmetry is not established until after fertilization, the unfertilized egg may be regarded as radially symmetrical.

If there were a complete localization of the mesenchyme-forming material at the vegetal pole of the *Lytechinus* egg, transection of the egg in the equatorial plane, thus into equal portions, should give one fragment (vegetal) which would develop into a larva with mesenchyme, and one fragment (animal) that, if it developed into a larva, should be without mesenchyme.

If the unfertilized egg is radially symmetrical, section made from pole to pole along the axis, dividing the egg into approximately equal portions, should give fragments both of which might develop into larvæ with mesenchyme. If the plane of symmetry were predetermined and the unfertilized egg were bilateral the same expectation would hold so far as the formation of mesenchyme is concerned.

Sections made at random, if there were complete localization of mesenchyme-forming material in the unfertilized egg, should give larvæ like those mentioned above, and in addition might be expected to give larvæ that differed in amount of mesenchyme, depending on whether the mesenchyme-forming material had been divided evenly or unevenly.

Sections of these three kinds were made. Those made in a plane parallel to that of the equator, *i. e.*, a plane at right angles to the direction of the axis, we may call horizontal; those made in any plane parallel to the direction of the axis and therefore at right angles to the plane of the equator may be called vertical; those made without orientation of the egg may be called random sections.

Our results showed very quickly that the animal and vegetal halves, the halves obtained by vertical section, and the halves obtained by random

sectioning, all gave rise to blastulæ with mesenchyme, which developed in turn to plutei. They afforded a demonstration of the absence of complete segregation of mesenchyme.

These results forced us to an examination of the comparative cleavage of the fragments, and raised the question as to whether a preliminary formation of micromeres must precede the formation of the mesenchyme.

The matter therefore presented itself in this form. If there were a complete localization of the micromere-forming material at the vegetal pole of the egg, a horizontal section should give one fragment (animal) which would form no micromeres, and one fragment (vegetal) which would form 4 micromeres. A vertical section, if through the poles, should give two fragments which would form the same number of micromeres on each piece; four on each, if each cleaved as a whole egg; two on each, if each cleaved as a half

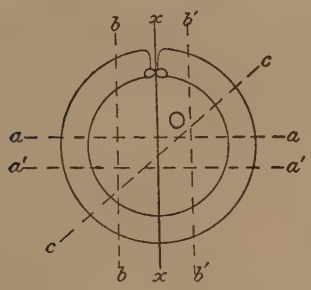


Fig. 1

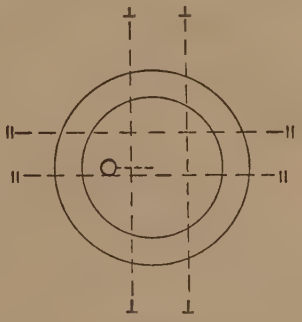


Fig. 2

egg. If the cleavage of the fragments were that of proportionate parts of the whole, the number of micromeres on each piece should vary with the degree of inclination of the plane of section to the axis of the egg (fig. 1).

Before we began our work in 1926, through the kindness of Professor T. H. Morgan and Mr. M. H. Harnly, we had the opportunity of reading the manuscript of Mr. Harnly's paper *Localization of the Micromere Material in the Cytoplasm of the Egg of Arbacia*, which has since been published in the Journal of Experimental Zoology, vol. 45, 1926.

Harnly reached the important conclusion that "the cleavage patterns of the two halves were directly dependent upon the plane of division in relation to the position of the nucleus." He found that when an egg, in which the nucleus lay at some distance from the center, was divided into approximate halves along a plane forming right angles with a line connecting the nucleus with the center of the egg, the nucleated fragment divided into the usual number of mesomeres, macromeres and micromeres at the fourth cleavage, but the non-nucleated fragment divided into two tiers of eight cells. On the other hand when the eggs were divided "along a plane perpendicular or nearly

perpendicular to the plane previously used," the micromeres were formed in both fragments in a high percentage of instances (fig. 2).

His results were so clear-cut that we investigated very thoroughly the development of *Lytechinus* fragments obtained by dividing eggs along a plane forming right angles with the line connecting the nucleus with the center of the egg, as well as that of fragments obtained by dividing an egg along a plane parallel to the line connecting the nucleus with the center of the egg.

Toward the end of our work we also found it desirable to make sections along a plane cutting the egg obliquely with reference to the axis (fig. 1 c-c).

The results that we present then are based on six types of section.

1. Horizontal.
2. Vertical.
3. Random.
4. At right angles to a line connecting the nucleus with the center of the egg.
5. Parallel to the line connecting the nucleus with the center of the egg.
6. Diagonal.

UNFERTILIZED EGG OF LYTECHINUS

A detailed consideration of the unfertilized egg of *Lytechinus* is unnecessary. There are a few points, however, which we found it desirable to establish by our own observation, which must be mentioned.

It is well known that this egg, like the egg of other regular sea-urchins, is surrounded by a layer of jelly whose existence may be demonstrated readily by placing the eggs in a suspension of Chinese ink in sea-water. The micropyle is most readily shown when the eggs are dropped into the suspension of ink without previous washing. Even a short period of washing may swell the jelly so as to block the micropyle and prevent the entrance of the particles of ink. With washed eggs one must sometimes examine many eggs before finding one that is satisfactory. In eggs which are dropped into the suspension without previous contact with water the micropyle may be seen in practically every egg. After some failure in the attempt to fertilize eggs cut in their jelly it was found that if the fragments were well washed immediately after cutting, their viability was not impaired.

Large numbers of eggs were examined to see whether the position of the micropyle was really an indication of the position of the animal pole. It was regarded as possible that the egg might rotate in its jelly and in that manner make the micropyle of uncertain value as a landmark. No evidence of rotation was found.

Careful examination showed the polar bodies lying at the base of the micropyle. In one instance only were they out of line as much as five degrees. The fact was verified that the first two cleavage planes cross each

other at right angles at the animal pole as marked by the position of the polar bodies and the micropyle.

There is a very noticeable difference between different lots of eggs in their viscosity and in the character of their membrane. It was by no means rare to find an individual whose eggs could not be cut. The eggs would burst as soon as the needle was brought into contact with them. It was our custom to prepare eggs from several individuals and use those which stood up best under treatment. After the use of eggs in their jelly was begun, it was found that a given lot was good for the work of about one hour.

Upon insemination of the entire egg, fusion of male and female nuclei takes place in from 7 to 8 minutes after insemination. The fusion may occur as early as 5 and as late as 10 minutes after the addition of the sperm suspension. Membrane formation is complete in from 1 to 1½ minutes after insemination. The spermatozoön enters, and the male nucleus, preceded by its aster, moves toward the female nucleus. Our observations confirm those of Wilson that in this egg "the meeting point of the pronuclei rarely if ever coincides with the ultimate position of the fusion-nucleus and the cleavage amphiaster and is sometimes far from it." (*The Cell*, 1925, page 425.)

During observations by one of us in 1925 it was found that about 10 per cent of the eggs of one individual were bi-nucleate. The separate nuclei were not all of the same diameter; they were distinctly smaller than the nucleus in the uninuclear eggs. Repeated observations were made on the fertilization of these eggs. The spermatozoön entered at any point on the surface. In some instances the sperm nucleus moved through a distance equal to fully half the diameter of the egg, not through the center, but directly toward the nearer of the egg-nuclei, which were eccentric in position. When the rays of the sperm aster came into contact with this nearer egg-nucleus, the egg-nucleus moved quickly toward the sperm-nucleus. Before fusion between these had occurred the more distant egg nucleus moved suddenly, as though it had been drawn in a current, to the proximal nucleus, came into contact with it, and the three fusing nuclei then moved toward their final position near the center of the egg, where the fusion became complete. This whole process of fertilization was completed in 6 minutes after insemination.

Eggs of this kind are similar to those of *Parechinus* discussed by Boveri ('18). Two individuals having many eggs of this kind were observed by us during the summer of 1926. An egg from one of these was cut so that there was a "partial nucleus" in each fragment (No. 136). Both fragments became fertilized and developed to late cleavage.

FRAGMENTATION OF THE EGGS

Great care was taken to avoid flattening and compression of the eggs. When they were placed in the drop on the slide and water withdrawn, care was taken that the eggs were not compressed and flattened by the surface film. The cut was upward toward the under side of the cover-slip.

If the eggs were very much compressed before cutting it was found that the fragments did not round up well. The fact has been mentioned that when the cutting was done slowly, with a needle of proper sharpness, the superficial layers of the egg were brought together, leaving no free wound surface.

Some of the most informing observations were made on fragments that were completely severed from one another, but that were allowed to remain in contact with each other along the surface of section. Eggs operated on in this way were particularly valuable in showing the relation of the cleavage planes to the surface of section, and the relation of the micromeres to the cleavage planes.

Fragments severed and allowed to remain in contact may fuse. On one occasion we attributed the fusion in several preparations to the jar caused by the dropping of a heavy packing box on the floor. Fusion occurred, however, even when no reason could be assigned, and was much more troublesome with some lots of eggs than with others.

In 1926 during the first two weeks of work, for unknown reasons, but possibly due to an unfavorable condition of the eggs, results were exceedingly disappointing. Very few pairs of fertilized fragments would develop. Then for the next three weeks everything went as smoothly as could be desired and the study of species-fertilized fragments was brought to a satisfactory conclusion.

FERTILIZATION OF EGG FRAGMENTS

During the latter half of work in 1926 the operator handed the observer, with the watch-glass containing the fragments, a sketch of the egg before cutting, showing the position of the nucleus and the location of the plane of section. The observer then located the fragments in the watch-glass, and sketched them in order to have a record of the amount that they had rounded up, so that the relation of the cleavage planes to the surface of section could be checked. They were then inseminated, during this season from 5 to 10 minutes after cutting.

In the fragments the fertilization membrane formed in the same time as on the entire egg. If the fragment had not rounded up well, *i.e.*, if there was a broad, flat surface of section, the membrane was well separated only on the convex surface and along the rim or edge of the flat surface.

The union of the nuclei in the nucleated fragment was as in the entire egg. In the non-nucleated fragment the sperm nucleus moved directly to the

center of the fragment. One might say that it had reached a position of equilibrium in the fragment, but having made the statement one is still without an explanation of the fact.

The development of the nucleated fragment is usually more rapid than the non-nucleated. The cleavage amphiaster may appear in an oblique position, and then rotate until it comes to lie in the long axis of the fragment; or it may appear and lie in the long axis from the beginning.

There were a few exceptions to this general behavior. In some fragments the amphiaster appeared in the short axis of the fragment, and during the progress of mitosis the fragment gradually broadened until its axes became nearly equal, the plane of division being in the original long axis of the fragment.

In the entire egg the first cleavage amphiaster lies horizontally in the cell at a level slightly above that of the equator. In fragments obtained by horizontal section the cleavage amphiaster forms in the long axis of the fragment, and thus has its original orientation with respect to the major axis of the egg. In fragments obtained by vertical section, when the amphiaster is lying in the long axis of the fragment, its position is at right angles to the position it would have had in the whole egg.

In the 1926 material in Nos. 256, 257, 260, 261 and 262, all obtained by vertical section of eggs of the same female, the first cleavage amphiaster appeared in the short axis of one or both fragments. In No. 257 the cell changed form about the spindle in each fragment; in Nos. 256, 260, 261 and 262 the spindle rotated until it was in the long axis of the cell at the time of division and the first two divisions were at right angles to the plane of section. These cases are considered in detail on page 22.

Usually the initial development of the nucleated fragment is slower than that of the whole egg, and that of the non-nucleated fragment slower than that of the nucleated fragment. Specific cases are convincing and important.

No. 3 Series of 1924 (fig. 3, a to i).
Fragments inseminated $11^h\ 56^m$.
Nucleated fragment.
Beginning constriction $12^h\ 37^m$.
Complete 2 cell $12^h\ 38^m$.
Time between insemination and complete division 42 minutes.
Non-nucleated fragment.
Beginning constriction $12^h\ 50^m$.
Complete 2 cell $12^h\ 55^m$.
Time between insemination and complete division 59 minutes.

No. 21 1924
Fragments inseminated $2^h\ 22^m$.
Nucleated fragment. Complete 2 cell $3^h\ 15^m$=53 minutes.
Non-nucleated fragment. Complete 2 cell $3^h\ 30^m$=68 minutes.

No. 38 1924
Fragments inseminated $2^h\ 30^m$.
Nucleated fragment. Complete 2 cell $3^h\ 25^m$=55 minutes.
Non-nucleated fragment complete 2 cell $3^h\ 45^m$=75 minutes.

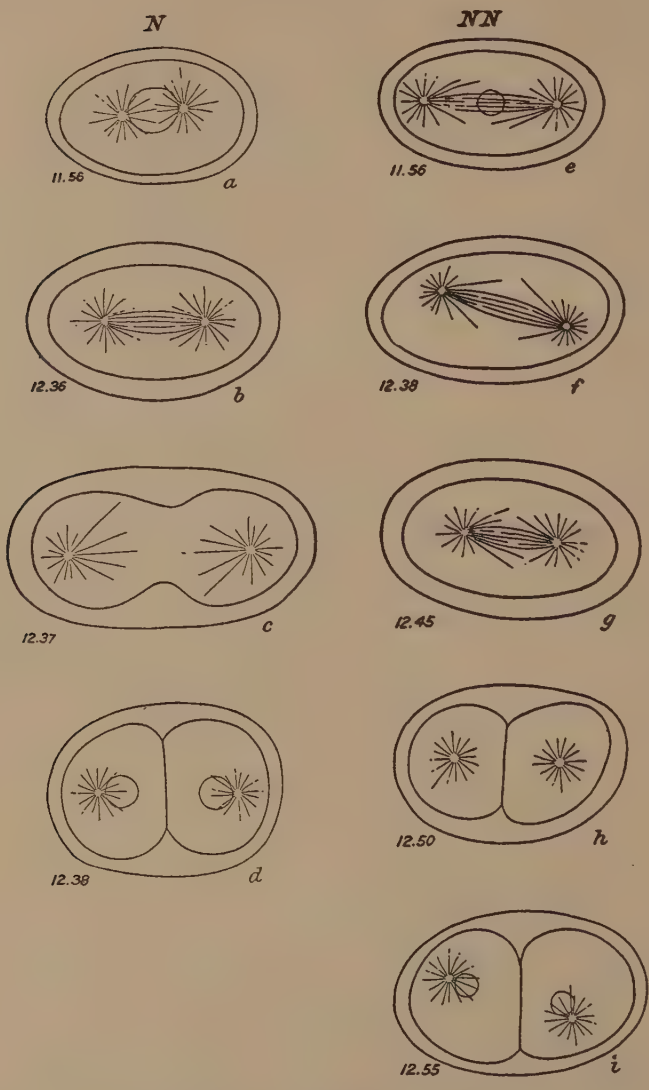


Fig. 3—Rate of development of nucleated and non-nucleated fragments

No. 137 1926 Relative volume N^1 : NN=1 : 1.
Fragments inseminated 10^h 25^m.
Nucleated fragment. Complete 2 cell 11^h 05^m=40 minutes.
Non-nucleated fragment. Complete 2 cell 11^h 16^m=51 minutes.

No. 211 1926 Relative volume N : NN=1 : 4.
Fragments inseminated 10^h 20^m.
Nucleated fragment.
Complete 2 cell 11^h 10^m=40 minutes.
Non-nucleated fragment. Complete 2 cell 11^h 21^m=61 minutes.

No. 220 1926 Relative volume N : NN=3 : 1.
Fragments inseminated 12^h 55^m.
Nucleated fragment. Complete 2 cell 1^h 34^m=39 minutes.
Non-nucleated fragment. Complete 2 cell 2^h 08^m=73 minutes.

No. 223 1926 Relative volume N : NN=1 : 2.5
Fragments inseminated 1^h 52^m.
Nucleated fragment. Complete 2 cell 2^h 36^m=44 minutes.
Non-nucleated fragment. Complete 2 cell 3^h 04^m=72 minutes.

No. 278 1926 Relative volume N : NN=1 : 5.
Fragments inseminated 10^h 35^m.
Nucleated fragment. Complete 2 cell 11^h 11^m=36 minutes.
Non-nucleated fragment. Complete 2 cell 11^h 18^m=43 minutes.

No. 279 1926 Relative volume N : NN=10 : 1.
Fragments inseminated 10^h 53^m.
Nucleated fragment. Complete 2 cell 11^h 35^m=42 minutes.
Non-nucleated fragment. Complete 2 cell 11^h 47^m=54 minutes.

When the sea-water was at a temperature of 29.5° C., with a whole egg the complete 2-cell stage was reached 35 minutes after insemination. With a given pair the nucleated fragment is from 1 to 20 minutes slower than the whole egg in completing its first division, the non-nucleated fragment from about 7 to 40 minutes slower than the whole egg. In the few examples given it may be seen that the difference between the two fragments of a given egg varied from 7 to 40 minutes. While the relative volumes given are approximate, they are sufficiently accurate to show that the whole explanation does not lie in difference in size of fragments. The difference in time can not be ascribed to delayed activation of one of the fragments, as the immediate formation of a fertilization membrane on both fragments affords a visible evidence of activation.

Tests showed that initial development was more successful when the fragments were not fertilized immediately after cutting. A period of 5 to 10 minutes between operation and insemination gave the best results. Nothing was gained by allowing either a longer or a shorter interval.

The usual sources of failure in development were failure in activation, polyspermy, cytolysis after an apparently normal start, and the formation of protrusions either at the time of insemination or at some time during

[1] Nucleated : Non-nucleated

cleavage. The substance of the egg may protrude slightly into the perivitelline space, or it may force its way out through the fertilization membrane. The transparent layer of ectoplasm may or may not be evident on these larger protrusions. The most usual site of protrusions was on the surface of section.

PERCENTAGE OF INITIAL DEVELOPMENT

The method used is one of great precision and is obviously of less violence than mass fragmentation. It offers the advantage of accurate information gained through careful orientation of material under high magnification.

The question may be put: Is the mortality of the operated material too high to give value to results observed after successful operation?

The purpose of the investigation required information on both members of a pair of fragments. The first indication of successful development is the formation of a fertilization membrane. If both members of a pair formed the membrane following insemination they were recorded as having begun development. If upon further examination it was found that some pairs in which the membrane was not well separated had been fertilized and had begun regular cleavage, they too were recorded as having begun development. In addition a very few single fragments that seemed of unusual promise were recorded and kept under observation even though the other member of the pair was obviously worthless. Their number is small, as may be seen by inspection of the tables in the appendix.

The data for each year will be presented separately, as there is nothing to be gained by combining the results of the two years.

Series of 1924

Total number of eggs cut and inseminated	122
Total pairs that developed at least as far as membrane formation	80
Percentage of initial development	65.7 p. ct.
Cleavage of both members of a pair	56
Percentage of total-cut cleaving	45.9 p. ct.

Series of 1926

Total number of eggs cut and inseminated	451
Total pairs that developed at least as far as membrane formation	285
Percentage of initial development	63.1 p. ct.
Cleavage of both members of a pair	197
Percentage of total-cut cleaving	43.6 p. ct.

It is also of interest to see whether the eggs cut in one plane were more successful in initial development than those cut in another.

A. Horizontal:

Total-cut	117
Total membrane-formation +	67
Percentage initial development	57.2 p. ct.

B. Vertical:

Total-cut	26
Total membrane-formation +	20
Percentage initial development	76.9 p. ct.

C. Perpendicular to line connecting nucleus with center of egg:
Total-cut 174
Total membrane-formation + ... 111
Percentage of initial development . . . . . 63.7 p. ct.

D. Parallel to line connecting nucleus with center of egg:
Total-cut 111
Total membrane-formation + ... 75
Percentage of initial development . . . . . 67.5 p. ct.

E Diagonal:
Total-cut 25
Total membrane formation+ 15
Percentage of initial development . . . . . 60 p. ct.

The numbers dealt with are too small for the figures to have any significance. From the data as they stand it would seem that the vertical sections had the best chance. When one compares these data with those of 1924, the figures seem less conclusive.

Series of 1924:
Cuts at random 40
Horizontal 31
Vertical 51

Total 122

Random initial development 23
Percentage of total random . . . . . 57.5 p. ct.
Horizontal initial development 22
Percentage of horizontal cuts . . . . . 70.9 p. ct.
Vertical initial development 35
Percentage of vertical cuts . . . . . 68.6 p. ct.

CLEAVAGE PATTERN OF THE WHOLE EGG AND OF THE FRAGMENTS

Cleavage in the *Lytechinus* egg is of the type common to the eggs of regular sea-urchins. The first two cleavages are meridional and the third, dividing the egg into 8 cells, the upper four of which are usually slightly smaller than the lower 4, is horizontal. The egg does not pass directly from an 8- to a 16-cell stage, but passes first into a 12-cell stage by a slightly oblique division of the four cells of the vegetative half of the egg. This is the division by which the 4 micromeres are cut off from the vegetative blastomeres at the vegetal pole of the egg.

The egg then passes into the 16-cell stage by the meridional division of the 4 cells of the upper half of the egg. The amphiaster in each of these cells is horizontally placed, but the cells shift in position as they are forming, so that 4 of them lie at a slightly higher level than the other 4. Boveri ('01), in describing the relative sizes of the cells of the 16-cell stage in *Paracentrotus*, designated them as 8 mesomeres, 4 macromeres and 4 micromeres, and this is now the accepted nomenclature for this stage of cleavage of the eggs of sea-urchins.

We have called the cleavage by which the micromeres are formed, the fourth cleavage. In designating this cleavage fourth and that forming the mesomeres fifth some violence is done to the tradition that the fourth cleavage gives rise to a 16-cell stage and the fifth to a 32-cell stage. Our fifth cleavage may be regarded as a delayed part of the fourth by any one to whom the idea of a fifth cleavage giving rise to a 16-cell stage seems inconceivable.

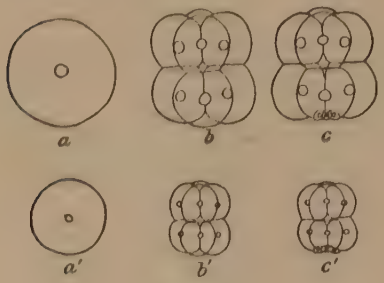


Fig. 4

FIG. 4—Cleavage of nucleated (*a*) and non-nucleated (*a′*) fragments, both fragments having become spherical; *b* and *b′*, 8-cell stage; *c* and *c′*, 12-cell stage.

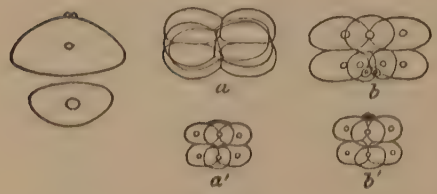


Fig. 5

FIG. 5—Horizontal section; (*a*) non-nucleated animal fragment and (*a′*) nucleated vegetal fragment, both in 8-cell stage; (*b*) non-nucleated animal fragment and (*b′*) nucleated vegetal fragment, both in 12-cell stage. In (*b*) one of the macromeres has divided equally instead of forming a micromere. Neither of the fragments has become spherical.

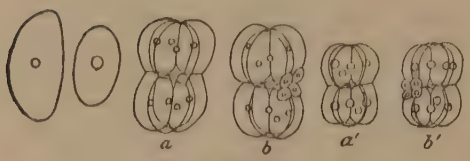


Fig. 6

FIG. 6—Vertical section; (*a*) non-nucleated and (*a′*) nucleated fragment, both in 8-cell stage; (*b*) non-nucleated and (*b′*) nucleated fragment, both in 12-cell stage.

Neither of the fragments has become spherical.

Two things should be kept in mind. First, the fact that the planes of division by which the micromeres are formed are oblique with reference to the axis of the egg, while the planes of division in the upper cells start as meridional furrows. Second, the fact that the micromeres are usually completely formed before the cells of the animal half have divided. In the experimental material when the cells of the 8-cell stage were equal in size and all divided equally to give first a 12-cell and then a 16-cell stage without micromeres, it was therefore possible to distinguish between "mesomeres" and "macromeres" by noting the relative time of formation of these cells.

When it was found that mesenchyme developed in larvæ obtained from fragments derived by cutting along any plane, it seemed desirable to follow the cleavage of the fragments in detail and to observe in these the resemblance to and difference from the characteristic cleavage of the entire egg.

What would be the direction of the planes of cleavage in halves obtained by horizontal transection? Would the animal half form micromeres? What would be the direction of the planes of cleavage in halves obtained by vertical section? Would both of these form four micromeres or would each form two? If the plane of section were slightly oblique to the axis of the egg would one half form 1 micromere and the other 3, or one none and the other 4? Would the proportionate size of fragments have any influence on the character of cleavage?

It was when we began to try to answer questions of this sort that it was found essential to make the observations continuous in order to trace the origin and fate of each cell. To do this it was necessary to make the observations on stages later than the third cleavage, under high power.

If the fragments round up perfectly after fragmentation it is impossible to reach conclusions concerning the relation of planes of cleavage to the surface of the section (fig. 4). If the fragments, however, round up incompletely, showing a lesser convexity on the surface of section than on the original outer surface (figs. 5 and 6), or if two fragments are allowed to remain in contact after the operation, it is possible to make observations that are thoroughly conclusive, provided these observations be made continuously and under sufficient magnification.

Early in our work of 1924 during the study of a pair of fragments obtained by vertical section (No. 26, fig. 7), and which remained in contact so that the original relationship of the parts was known, it was observed that the first plane of cleavage was at right angles to what it would have been in the entire egg, that the first two planes of cleavage were at right angles to the plane of section, and that the micromeres were formed on the cut surface at the point of intersection of the first and second cleavage planes. The possible significance of the fact that the first cleavage in the fragment was at right angles to what it would have been in the entire egg was so apparent that the observation was extended on every pair of fragments practicable. As has been stated, those which could serve as a basis for observation had either to be fragments that did not round up or that were adhering to each other along the plane of the cut.

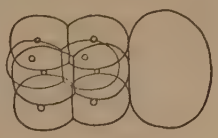


Fig. 7

The total number of pairs available for this purpose is 61; 16 in the series of 1924; 45 in the series of 1926. Their distribution is as follows:

1. Plane of section unknown; all series of 1924: Nos. 3, 4, 21, 48, 49, 52; total 6.
2. Plane of section horizontal; Series of 1924: Nos. 54, 70; Series of 1926: Nos. 140, 198, 203, 204, 206, 217, 223, 226, 231, 237, 238, 242, 252, 253, 269; total 17.

3. Plane of section vertical; Series of 1924: Nos. 26, 30, 34, 38, 56, 64, 69, 73; Series of 1926: Nos. 256, 257, 258, 260, 261, 262, 264, 266, 268; total 17.
4. Plane of section right angles to line connecting nucleus with center of egg; all Series of 1926: Nos. 21, 22, 23, 24, 49, 53, 54, 56, 57, 77, 92, 93, 176; total 13.
5. Plane of section parallel to line connecting nucleus with center of egg; all Series of 1926: Nos. 29, 109, 124, 174, 178; total 5.
6. Plane of section diagonal to axis of egg; all Series of 1926: Nos. 272, 280, 283; total 3.

In the fragments obtained by horizontal section the amphiasters of the first division always appeared in the long axis of the cell, *i.e.*, in a plane parallel to that in which it would have appeared in the whole egg. Only one unusual feature appeared; in No. 242 the plane of second cleavage was parallel instead of at right angles to the surface of section, the micromeres forming at one of the intersections of the first and second planes, thus not on the cut surface (fig. 8).

Fig. 8

In the fragments obtained by vertical section, those from 6 eggs require careful consideration, viz. No. 38 (1924), and Nos. 256, 257, 260, 261 and 262 (1926). In the non-nucleated fragment of No. 38 the amphiaster appeared in the short axis of the fragment; the fragment then gradually broadened until its axes became nearly equal, division being in a plane parallel to the original axis of the egg. The amphiaster in the nucleated fragment formed in the long axis of the fragment and division as usual was at right angles to the surface of section.

In both the nucleated and non-nucleated fragments of No. 256, the first cleavage amphiaster appeared in the short axis of the fragments. The amphiaster then rotated and division was at right angles to the cut surface.

In both the nucleated and non-nucleated fragments of No. 257, the amphiaster appeared in the short axis and the cell then changed form around it, division being parallel to the original surface of section.

The amphiaster appeared in the long axis of the nucleated fragment of No. 260 and division was at right angles to the plane of section. The amphiaster in the non-nucleated fragment appeared in the short axis, rotated, and division was at right angles to the plane of section.

In both the nucleated and non-nucleated fragments of No. 261, the amphiaster appeared in the short axis, rotated, and division was in a plane at right angles to that of section.

In both the nucleated and non-nucleated fragments of No. 262 the amphiaster appeared in the short axis. The actual division of the fragments was not observed.

The fragments of these 6 eggs stand in contrast to those of the remaining 11 of the vertical section series here under consideration, in that in one or both fragments the amphiaster appeared in the short axis of the fragment instead of in the long axis. They differ among themselves in that in three

fragments the contents rearranged itself around the amphiaster and division was parallel to the surface of section, while in the others the amphiaster rotated and division was at right angles to the plane of section. In one of the vertical series, No. 69 (1924), the second division was parallel to the plane of section in the nucleated fragment.

Finally, in three of the eggs whose plane of section was unknown, Nos. 21, 48 and 52 (all 1924), the facts suggested that these unknown sections were probably vertical. The amphiaster in both the nucleated and non-nucleated fragments of No. 21 was formed in the short axis and rotated before division. The amphiaster formed in the long axis of the nucleated fragment of No. 48; it formed in the short axis of the non-nucleated fragment and rotated until it came to lie in the long axis. In both fragments division was at right angles to the plane of section. In No. 52 (fig. 9) the amphiaster formed in the short axis, but no further data can be given as this fragment was kept so long under a cover-slip for study that its development stopped. The diagonal sections, and those obtained by cutting the egg in a plane either at right angles to or parallel to the line connecting the nucleus with the center of the egg, supported the observation that the first two planes of cleavage were at right angles to the cut surface.

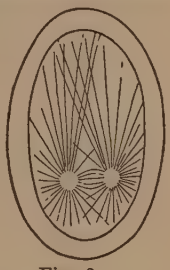


Fig. 9

The non-nucleated fragment of No. 38 (1924) and the two fragments of No. 257 (1926) stand as exceptions to the observed fact that in fragments the plane of the first cleavage is at right angles to the plane of section.

The fact that, in fragments obtained by vertical section, the amphiaster often appears in the short axis of the fragment suggests the persistence of an original organization relative to the axis of the egg. We will consider this suggestion more fully in a section on polarity after presenting our material on micromeres.

A STUDY OF THE FORMATION OF MICROMERES IN FRAGMENTS OF SEA-URCHIN EGGS

It has already been stated that this work was begun with the assumption that a material destined to give rise to micromeres, and these in turn to mesenchyme, was localized in the vegetative half of the unfertilized *Lytechinus* egg in the region surrounding the vegetal pole.

When investigation showed that mesenchyme was formed in larvæ derived from fragments in which there was no material from the vegetative half of the egg, the material presented itself in a new aspect. Could any fragment form micromeres? Were micromeres necessarily antecedent to mesenchyme?

Near the close of our work in 1924, since adequate information was still lacking, the final two days were devoted to the study of the formation of

micromeres. Up to that time in every pair in which observations were satisfactory, the cleavage of one of the fragments followed the pattern of the whole egg, while in the other the division which should have given rise to the micromeres was an equal division of the cells of one half of the fragment shortly after the equal division of the four cells of the other half of the fragment.

In nearly the last pair of fragments studied, No. 77, from an egg cut vertically, the nucleated fragment formed 4 micromeres; the non-nucleated fragment also seemed to produce 4 micromeres but the observation could not be completed satisfactorily as the fragment was injured in manipulation. We did not have full confidence in the observation and could not regard it as satisfactory. This one doubtful observation made it evident that the problem could not be regarded as solved.

Through the use of improved methods of observation used during the summer of 1926 we are able to present thoroughly dependable data on the first four cleavages of one or both members of a pair from 158 out of 285 pairs of fragments that were successfully activated. This includes complete and satisfactory observations on both the nucleated and non-nucleated fragments of 80 eggs. The total number of eggs cut during 1926 was 451. These were sectioned as follows:

A. Horizontal	$115 + 2^1 = 117$
B. Vertical	$25 + 1^1 = 26$
C. Right angles to line connecting nucleus with center of egg	174
D. Parallel to line connecting nucleus with center of egg	111
E. Diagonal	25
F. Random	1

Complete data on one or both fragments were obtained from 158 eggs.

A. Cut horizontal; 43 instances. 24 of these being on both nucleated and non-nucleated fragments; 16 on the nucleated but not the non-nucleated fragment; and 3 on the non-nucleated, but not the nucleated fragment.

B. Cut vertical; 12 instances. 8 of these being on both the nucleated and non-nucleated fragments; 4 on the nucleated but not the non-nucleated fragment.

C. Cut right angles to the line connecting the nucleus with the center of the egg; 46 instances. 30 of these being on both the nucleated and non-nucleated fragments; 12 on the nucleated but not the non-nucleated fragment; and 4 on the non-nucleated but not the nucleated.

D. Cut parallel to the line connecting the nucleus with the center of the egg; 44 instances. 28 of these being on both the nucleated and the non-nucleated fragments; 14 on the nucleated but not the non-nucleated fragment; and 2 on the non-nucleated but not the nucleated fragment.

E. Cut diagonal; 15 instances. 6 of these being on both the nucleated and non-nucleated fragments; 8 on the nucleated but not on the non-nucleated fragment; and 1 on the non-nucleated but not the nucleated fragment.

[1] No. 16 was cut in a plane at right angles to the line, etc., which was also a horizontal plane.

No. 110 was cut in a plane parallel to the line, etc., which was also a horizontal plane.

No. 39 was cut in a plane parallel to the line, etc., which was also a vertical plane.

The data may be analyzed in another way in view of the fact that the complete observations show that in some instances no micromeres are formed on either the nucleated or non-nucleated fragments.

A. Horizontal:
- No micromeres on either 2
- One or more micromeres on N. 25
- One or more micromeres on NN. 22

B. Vertical:
- No micromeres on either 1
- One or more micromeres on N. 10
- One or more micromeres on NN. 4

C. Right angles to line, etc.:
- No micromeres on either 5
- One or more micromeres on N. 33
- One or more micromeres on NN 27

D. Parallel to line, etc.:
- No micromeres on either 2
- One or more micromeres on N. 38
- One or more micromeres on NN. 20

A complete table showing all of the possible associations of micromeres that might occur is presented here for the purpose of showing the actual distribution of micromeres, case by case, as they fitted into their places in the table.

Abbreviations and Explanation of Tabulation

N, Nucleated
NN, Non-nucleated
The subscripts 0, 1, 2, 3 and 4 indicate:
- 0, no micromeres formed; each of the vegetative blastomeres divided equally.
- 1, 1 micromere formed; 3 of the vegetative blastomeres divided equally.
- 2, 2 micromeres formed; 2 of the vegetative blastomeres divided equally.
- 3, 3 micromeres formed; 1 of the vegetative blastomeres divided equally.
- 4, 4 micromeres formed.

$N^?$ nucleated fragment in which the character of cleavage was not determined.
$NN^?$ non-nucleated fragment in which the character of cleavage was not determined.

Example: N_0NN_0 means that no micromeres were formed on either the nucleated or non-nucleated fragment and that each of the vegetative blastomeres divided equally in the fourth cleavage. N_4NN_1 means that 4 micromeres were formed on the nucleated and 1 on the non-nucleated; *i. e.*, 1 of the vegetative blastomeres formed a micromere and the other 3 divided equally.

$N_3NN^?$ indicates that 3 micromeres were formed on the nucleated fragment, 3 of the vegetative blastomeres forming micromeres and 1 dividing equally, while the facts regarding the development of the non-nucleated fragment were not determined. The numbers 88, 92, 126, etc., are the recorded numbers of the pairs as they stand in our records of the Series of 1926. A full history of each case will be found in the appendix, reference to which should be made by the numbers indicated.

Distribution relative to principal axis of egg.

Vertical	Horizontal	Vertical	Horizontal
N_0 N N_0	N_0 N N_0	N_3 N N_3	N_3 N N_3
No. 268	No. 221	None	None
	230	N_3 N N_4	N_3 N N_4
		None	None
	Total 2	N_4 N N_0	N_4 N N_0
		No. 1	No. 202
N_0 N N_1	N_0 N N_1	137	
None	None		
N_0 N N_2	N_0 N N_2	Total 2	
None	No. 231		
	240	N_4 N N_1	N_4 N N_1
		None	None
	Total 2	N_4 N N_2	N_4 N N_2
		No. 258	None
N_0 N N_3	N_0 N N_3	N_4 N N_3	N_4 N N_3
None	No. 254	None	No. 214
N_0 N N_4	N_0 N N_4	N_4 N N_4	N_4 N N_4
None	No. 199	No. 257	No. 16
	201	264	242
	209	265	269
	237		
		Total 3	Total 3
	Total 4		
		N_0 N N_7	N_0 N N_7
N_1 N N_0	N_1 N N_0	No. 267	No. 203
None	None		204
N_1 N N_1	N_1 N N_1		206
None	None		224
N_1 N N_2	N_1 N N_2		227
None	None		251
N_1 N N_3	N_1 N N_3		
None	None		Total 6
N_1 N N_4	N_1 N N_4		
None	None	N_1 N N_7	N_1 N N_7
N_2 N N_0	N_2 N N_0	None	None
None	No. 229	N_2 N N_7	N_2 N N_7
	252	No. 262	No. 193
			222
	Total 2		
			Total 2
N_2 N N_1	N_2 N N_1		
None	No. 226	N_3 N N_7	N_3 N N_7
N_2 N N_2	N_2 N N_2	None	No. 216
None	No. 140		223
	200		228
	217		
			Total 3
	Total 3		
		N_4 N N_7	N_4 N N_7
N_2 N N_3	N_2 N N_3	No. 39	No. 215
None	None	259	220
N_2 N N_4	N_2 N N_4		238
None	No. 192		246
N_3 N N_0	N_3 N N_0		253
No. 266	None		
N_3 N N_1	N_3 N N_1	Total 2	Total 5
None	No. 235		
N_3 N N_2	N_3 N N_2	N_7 N N_0	N_7 N N_0
None	No. 195	None	None
	210	N_7 N N_1	N_7 N N_1
		None	None
	Total 2	N_7 N N_2	N_7 N N_2
		None	No. 233
		N_7 N N_3	N_7 N N_3
		None	No. 194
		N_7 N N_4	N_7 N N_4
		None	No. 247

Distribution relative to line connecting nucleus with center of the egg.

Perpendicular	Parallel	Perpendicular	Parallel
N_0 N N_0	N_0 N N_0	N_4 N N_0	N_4 N N_0
No. 88	No. 31	No. 135	No. 44
92	36		107
126			116
130			157
134			
Total 5	Total 2		Total 4
		N_4 N N_1	N_4 N N_1
N_0 N N_1	N_0 N N_1	None	No. 104
None	None	N_4 N N_2	N_4 N N_2
N_0 N N_2	N_0 N N_2	No. 142	No. 110
No. 133	None	153	
N_0 N N_3	N_0 N N_3		
None	None	Total 2	
N_0 N N_4	N_0 N N_4		
No. 74	None	N_4 N N_3	N_4 N N_3
N_1 N N_0	N_1 N N_0	None	No. 121
None	None	N_4 N N_4	N_4 N N_4
N_1 N N_1	N_1 N N_1	No. 16	No. 37
None	None	21	109
N_1 N N_2	N_1 N N_2	54	115
None	None	57	119
N_1 N N_3	N_1 N N_3	146	144
No. 187	No. 189	152	151
N_1 N N_4	N_1 N N_4	154	164
None	None	156	
N_2 N N_0	N_2 N N_0		
None	No. 43	Total 8	Total 7
N_2 N N_1	N_2 N N_1		
No. 149	No. 148	N_0 N N_7	N_0 N N_7
N_2 N N_2	N_2 N N_2	No. 96	No. 35
No. 89	No. 105	188	161
97	113		
173	155	Total 2	Total 2
183	178		
184		N_1 N N_7	N_1 N N_7
		No. 167	No. 191
Total 5	Total 4	No. 180	
N_2 N N_3	N_2 N N_3	Total 2	
None	No. 99		
N_2 N N_4	N_2 N N_4	N_2 N N_7	N_2 N N_7
No. 181	None	No. 176	No. 108
N_3 N N_0	N_3 N N_0		112
No. 169	No. 34		122
	174		171
	Total 2		Total 4
N_3 N N_1	N_3 N N_1	N_3 N N_7	N_3 N N_7
No. 65	No. 163	No. 158	No. 106
80		N_4 N N_7	N_4 N N_7
94		No. 15	No. 25
Total 3		24	27
		77	39
		145	40
N_3N N_2	N_3 N N_2	165	117
None	No. 118	170	141
N_3 N N_3	N_3 N N_3		
None	None	Total 6	Total 6
N_3 N N_4	N_3 N N_4		
None	None		

Distribution relative to line connecting nucleus with center of the egg.—(Continued.)

Perpendicular	Parallel	Perpendicular	Parallel
N_7 N N_0 None N_7 N N_1 None N_7 N N_2 No. 61 175 Total 2	N_7 N N_0 No. 32 N_7 N N_1 None N_7 N N_2 None	N_7 N N_3 None N_7 N N_4 No. 55 63 Total 2	N_7 N N_3 None N_7 N N_4 No. 185

Distribution relative to principal axis of egg.

Diagonal					
N_0 N N_0 0	N_1 N N_0 0	N_2 N N_0 0	N_3 N N_0 No. 279	N_4 N N_0 No. 275 282	N_7 N N_0 0
N_0 N N_1 0	N_1 N N_1 0	N_2 N N_1 0	N_3 N N_1 0	N_4 N N_1 0	N_7 N N_1 0
N_0 N N_2 0	N_1 N N_2 0	N_2 N N_2 0	N_3 N N_2 No. 258	N_4 N N_2 0	N_7 N N_2 0
N_0 N N_3 No. 278	N_1 N N_3 0	N_2 N N_3 0	N_3 N N_3 0	N_4 N N_3 0	N_7 N N_3 No. 281
N_0 N N_4 0	N_1 N N_4 0	N_2 N N_4 0	N_3 N N_4 0	N_4 N N_4 0 N_4 N N_5 No. 277	N_7 N N_4 0
N_0 N N_7 No. 276	N_1 N N_7 0	N_2 N N_7 No. 272 273 283 284 Total 4	N_3 N N_7 0	N_4 N N_7 No. 271 274 280 Total 3	

TABLE 1.—*Summary of distribution of micromeres.*

Class	I Vertical	II Horizontal	III Diagonal	IV ‖ to Line	V ⊥ to Line
N_0NN_0	1	2	0	2	5
N_0NN_7	1	6	1	2	2
N_0NN_2	0	2	0	0	1
N_0NN_3	0	1	1	0	0
N_0NN_4	0	4	0	0	1
N_1NN_7	0	0	0	1	2
N_1NN_3	0	0	0	1	1
N_2NN_0	0	2	0	1	0
N_2NN_7	1	2	4	4	1
N_2NN_1	0	1	0	1	1
N_2NN_2	0	3	0	4	5
N_2NN_3	0	0	0	1	0
N_2NN_4	0	1	0	0	1
N_3NN_0	1	0	1	2	1
N_3NN_7	0	3	0	1	1
N_3NN_1	0	1	0	1	3
N_3NN_2	0	2	1	1	0
N_4NN_0	2	1	2	4	1
N_4NN_7	2	5	3	6	6
N_4NN_1	0	0	0	1	0
N_4NN_2	1	0	0	1	2
N_4NN_3	0	1	0	1	0
N_4NN_4	3	3	0	7	8
N_4NN_5	0	0	1	0	0
N_7NN_0	0	0	0	1	0
N_7NN_2	0	1	0	0	2
N_7NN_3	0	1	1	0	0
N_7NN_4	0	1	0	1	2
Totals	12	43	15	44	46

Table 1 presents a summary of the distribution of micromeres that has been described in the preceding pages. The numbers appearing at the right of each class title indicate the frequency of that class. The sum of the frequencies is 160, instead of 158, for the reason that one of the pairs was obtained by a horizontal cut which was also a cut at right angles to the line connecting the nucleus with the center of the egg; while another of the pairs was obtained by a vertical cut which was also a cut parallel to a line passing from the nucleus to the center of the egg. Each of these pairs therefore appears in two columns.

Our study of the fragments during their early cleavage (to the expected 16-cell stage) showed five types following a normal 4-cell stage:

Type A. Normal 8 cell, and normal 16 cell with 4 micromeres.
Type B. Normal 8 cell, and then equal division of each cell, giving 16 approximately equal cells (fig. 10).
Type C. Normal 8 cell followed by the unequal division of one, and the equal division of the remaining 7 cells (fig. 11).
Type D. Normal 8 cell followed by the unequal division of two, and the equal division of the remaining 6 cells (fig. 5b).
Type E. Normal 8 cell followed by the unequal division of three, and the equal division of the remaining 5 cells (fig. 12).

Fig. 10 Fig. 11 Fig. 12

Fig. 10—View of 12-cell stage from side.
Fig. 11—View of 12-cell stage from vegetal pole.
Fig. 12—View of 12-cell stage from vegetal pole.

Driesch (1897) found that fertilized egg fragments usually cleaved normally if they were large. He described five types of modified cleavage. Our observations on our Types A to E, and on modifications of these types due to differences in cell sizes in the 4- and 8-cell stages, afford a complete confirmation of Driesch's observations on cleavage-pattern of egg-fragments obtained by mass fragmentation.

A CRITICAL CONSIDERATION OF THE RESULTS AND OF THE POSSIBLE LOCUS OF MICROMERE-FORMING SUBSTANCE IN THE UNFERTILIZED EGG

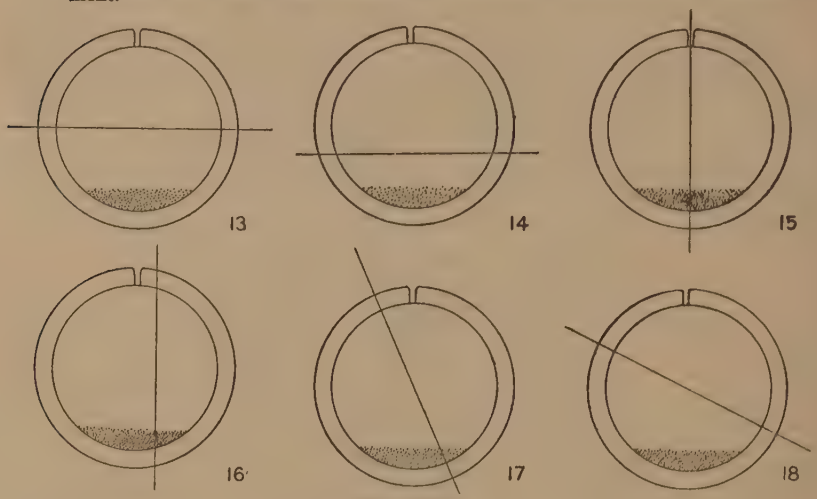
I. Localization of micromere-forming substance at the vegetal pole (figs. 13 to 18).

1. Horizontal cut.

The animal fragment of the egg should be expected to form no micromeres; the vegetal fragment should be expected to form 4 micromeres (figs. 13 and 14).

2. Vertical cut.

(a) If the plane of section passed through both poles (fig. 15), each half should be expected to form the same number of micromeres, the number depending on whether the fragments cleaved as a whole egg or as a half. If each half cleaved as a whole egg one should expect to find 4 micromeres on each; if each half cleaved as a half egg one should expect to find 2 micromeres on each fragment.



Figs. 13-18

(b) If the plane of section were parallel to the axis of the egg and divided the egg into unequal portions (fig. 16), one might expect 4 large micromeres on the large fragment and 4 small micromeres on the smaller fragment if the fragments cleaved as a whole egg, or a varying number of micromeres if the fragments cleaved as relative parts of an egg.

3. Diagonal cut (figs. 17 and 18).

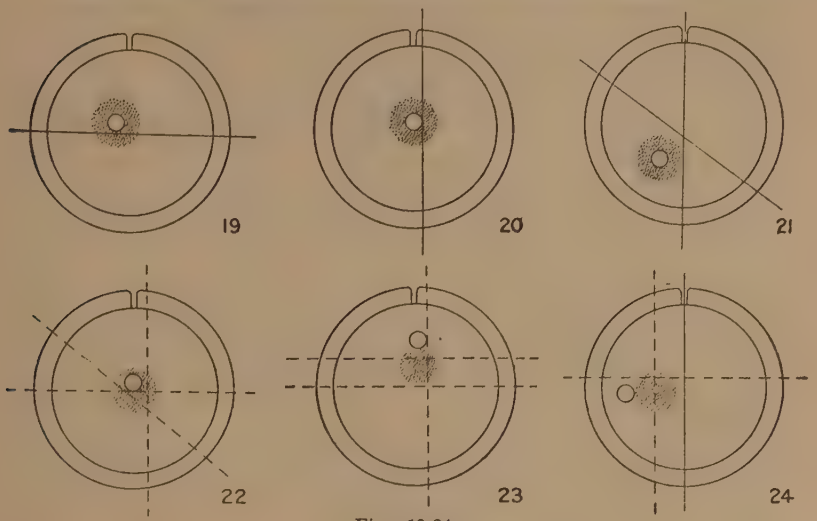
Many possible relative distributions of the micromere-forming substance from that given by a plane of section that just misses being horizontal, to one that just misses being vertical, may be imagined. The result of fragmentation in every instance would be one fragment that had no micromere-forming substance and one that had all of it; or one that had a smaller amount and one that had a greater; or both might have an equal amount.

II. Localization of the micromere-forming substance as a sphere surrounding the nucleus (figs. 19 to 22).

It is recognized that the nucleus may lie in any position in the cell. The results of section on distribution would differ in no wise from those which have been considered under the preceding headings. An eccentric position of the nucleus in such a sphere would offer no possibilities that have not been considered.

III. Localization of micromere-forming substance between the nucleus and the center of the egg (Harnly's Theory) (figs. 23 and 24).

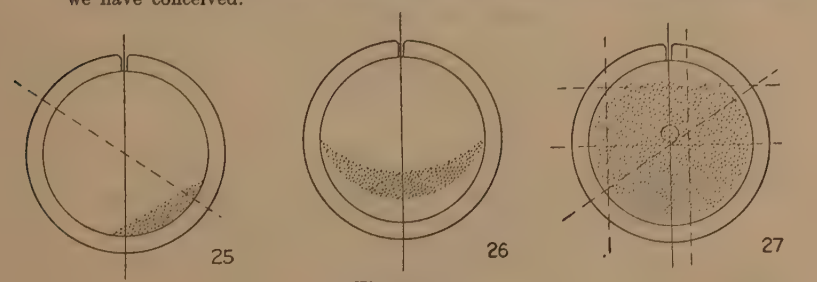
Every possible cut must either give one fragment that contains no micromere-forming substance and one that contains all of it, or two fragments that contain either equal or variable amounts of the micromere-forming substance.



Figs. 19-24

IV. Localization of the micromere-forming substance as a segment, whose surface might appear in the form of a crescent, the upper edge of this crescent being about 45° from the vegetal pole on the meridian dividing it equally. It would still be possible in cutting, to obtain one fragment containing micromere-forming material and one free from it; or two fragments containing it in equal or in varying amounts (figs. 25 and 26).

The existence of bilaterality must accompany such a localization as that here pictured, as indeed it may accompany any other of the localizations of which we have conceived.



Figs. 25-27

V. Distribution of micromere-forming substance throughout more than half the egg (fig. 27).

The results of fragmentation with this distribution could differ from those that we have considered only in the fact that it would not be possible to obtain an entire half that would be free from micromere-forming material.

But no matter what the localization or distribution, it would not be possible to divide the egg in such manner that neither fragment would contain micromere-forming material.

An examination of our records shows that from horizontal sections, sections made at right angles to, and sections made parallel to the line connecting the nucleus with the center of the egg, we sometimes obtained pairs of fragments neither of which formed micromeres.

The fact remains, however, that from every type of section of the *Lytechinus* egg that we made we were able to obtain larvæ with mesenchyme.

The conclusion that the formation of primary mesenchyme is not dependent on the formation of micromeres naturally follows.

Fragments that give rise to no micromeres, or to one, or to two, or to three, or to four during their cleavage may all give rise to larvæ with mesenchyme.

Size of fragments or the region of the surface involved seems to be without influence. A fragment less than 1/20 the volume of the entire egg, taken from near the animal pole, the region farthest from that in which the localized micromere-forming substance has been supposed to lie, may give rise to a normal larva with mesenchyme.

Mesenchyme may arise in larvæ in which no micromeres have been formed. The fact, however, that the cells formed by the fourth cleavage may be equal in size does not necessarily mean that four of them may not be analogous to the micromeres of the normal egg.

The experiments with *Lytechinus* have shown that primary mesenchyme may develop in larvæ arising from any spherical segment having 1/20 the volume of the egg. They show that there is appreciably no more mesenchyme in larvæ arising from the vegetal half of the egg than there is in larvæ arising from the animal half. These may both, so far as one can tell, be normal in every respect save that of size.

The experiments have indicated that prior to fertilization there had not been a segregation of mesenchyme-forming substance, but that a mesenchyme-endoderm-forming substance had a uniform distribution throughout about 19/20 of the egg. The animal polar cap seems to contain only ectoderm-forming material.

CONSIDERATION OF CASES IN WHICH NEITHER FRAGMENT FORMED MICROMERES AND THOSE IN WHICH BOTH FRAGMENTS FORMED FOUR MICROMERES

These two groups constitute the extremes; on the one hand a lack of micromeres on both fragments and on the other 4 micromeres on each.

The most interesting comparison is between the groups in which the orientation of the egg was relative to the principal axis, *i.e.*, the horizontal and the vertical groups.

Horizontal		Vertical
$N_0 NN_0$		$N_0 NN_0$
No. 221	Animal fragment nucleated	No. 268
No. 230	Animal fragment nucleated	
Total 2		Total 1
$N_4 NN_4$		$N_4 NN_4$
No. 16	Position of nucleus not recorded	No. 257
No. 242	Animal fragment nucleated	No. 264
No. 269	Vegetal fragment nucleated	No. 265
Total 3		Total 3

Unfortunately the vertical series group is the smallest of those under consideration. There were only eight instances in which observations on both members of a pair were complete. The fact, previously noted, that there seems to be a persistence of the original polarity of the egg, as shown by the tendency of the first cleavage amphiaster to form in the short axis of the fragment, makes a close scrutiny of the entire vertical group desirable.

We find that these 8 cases have an interesting distribution, 3 being in the $N_4 NN_4$ group. (There were 3 out of 8 in the Vertical Series and 3 out of 24 in the Horizontal Series.) Further, 2 of the Vertical Series are in the $N_4 NN_0$ group, 2 in the $N_4 NN^?$ group and 1 in the $N_4 NN_2$ group; *i.e.*, of the 12 instances in which we have complete data on one or both fragments, in 8 cases the nucleated fragment formed 4 micromeres.

Turning now to the Horizontal Series, we find that there are 16 instances in which either the animal or the vegetal fragment formed 4 micromeres. These are:

$N_0 NN_4$ No. 199 Animal fragment nucleated and smaller.
$N_0 NN_4$ No. 201 Animal fragment nucleated, fragments of equal size.
$N_0 NN_4$ No. 209 Animal fragment nucleated and smaller.
$N_0 NN_4$ No. 237 Animal fragment nucleated and smaller.
$N_2 NN_4$ No. 192 Animal fragment nucleated and larger.
$N_4 NN_0$ No. 202 Animal fragment non-nucleated and smaller.
$N_4 NN_3$ No. 214 Animal fragment non-nucleated and smaller.
$N_4 NN_4$ No. 16 Position of nucleus not recorded.
$N_4 NN_4$ No. 242 Animal fragment nucleated and larger.
$N_4 NN_4$ No. 269 Animal fragment non-nucleated and smaller.
$N_4 NN^?$ No. 215 Animal fragment non-nucleated and smaller.
$N_4 NN^?$ No. 220 Animal fragment non-nucleated and smaller.
$N_4 NN^?$ No. 238 Animal fragment nucleated and larger.
$N_4 NN^?$ No. 246 Animal fragment non-nucleated; slightly smaller.
$N_4 NN^?$ No. 253 Animal fragment nucleated; much larger.
$N^? NN_4$ No. 247 Animal fragment non-nucleated; very much larger.

This may be summarized:
Nucleated animal fragment . . . . . 4 micromeres in 3 instances.
Non-nucleated animal fragment . . . . . 4 micromeres in 6 instances.
Nucleated vegetal fragment . . . . . 4 micromeres in 2 instances.
Non-nucleated vegetal fragment . . . . . 4 micromeres in 6 instances.

The conclusion may be drawn that the presence or absence of the egg nucleus in the animal or vegetal fragment seems without significance on the occurrence of 4 micromeres.

SIZE OF FRAGMENT AND PRESENCE OR ABSENCE OF THE EGG NUCLEUS

Animal fragments nucleated and smaller, 4 micromeres	0
Animal fragments nucleated and larger, 4 micromeres	3
Animal fragments non-nucleated and smaller, 4 micromeres	5
Animal fragments non-nucleated and larger, 4 micromeres	0
Vegetal fragments nucleated and smaller, 4 micromeres	1
Vegetal fragments nucleated and larger, 4 micromeres	2
Vegetal fragments non-nucleated and smaller, 4 micromeres	2
Vegetal fragments non-nucleated and larger, 4 micromeres	3

The striking thing shown is that the smaller non-nucleated animal fragments seem as likely to produce 4 micromeres as do any of the vegetal fragments, large or small, nucleated or non-nucleated.

The following is a consideration of groups in which orientation of the egg was relative to a line connecting the nucleus with the center of the egg:

Right Angles to the Line:	Parallel to the Line:
$N_0 NN_0$	$N_0 NN_0$
No. 88	No. 31
No. 92	No. 36
No. 126	
No. 130	
No. 134	
Total 5	Total 2
$N_4 NN_4$	$N_4 NN_4$
No. 16	No. 37
No. 21	No. 109
No. 54	No. 115
No. 57	No. 119
No. 146	No. 144
No. 152	No. 151
No. 154	No. 164
No. 156	
Total 8	Total 7

The opportunity for a legitimate comparison seems to be good here. 13 out of 30 instances in which observations were complete on both fragments following cutting in a plane at right angles to the line are either in the $N_0 NN_0$ or $N_4 NN_4$ group; 9 out of 28 complete observations when transection was in a plane parallel to the line are in the $N_0 NN_0$ and $N_4 NN_4$ groups. The distribution is uniform except for the "Parallel" $N_0 NN_0$ class.

COMPARISON OF RESULTS FOLLOWING TRANSECTION OF LYTECHINUS EGGS, WITH REFERENCE TO THE POSITION OF THE NUCLEUS, WITH THOSE OF HARNLY ON ARBACIA

Our observations concerning the formation of micromeres on both fragments were complete for 30 eggs which were cut along a plane at right angles to the line connecting the nucleus with the center of the egg. In 5 of these 30 pairs no micromeres were formed on either fragment; in 21 of the 30 pairs one or more micromeres were formed on both the nucleated and non-nucleated fragments. 2 of the 4 remaining pairs (Nos. 74 and 133) had no micromeres on the nucleated fragment. One (No. 74) had 4 and the other (No. 133) had 2 micromeres on the non-nucleated fragment. Of the remaining 2 of the 30 pairs, one (No. 135) had 4 micromeres on the nucleated and none on the non-nucleated fragment, while the other (No. 169) had 3 micromeres on the nucleated and none on the non-nucleated fragment.

For convenience, these results from 30 pairs obtained by cutting the egg in a plane perpendicular to the line joining the nucleus with the center of the egg are grouped here:

N_0NN_0: Nos. 88, 92, 126, 130, 134; total, 5 pairs.
N_3NN_3: No. 187; N_2NN_2: No. 149; N_2NN_3: Nos. 89, 97, 173, 183, 184; N_2NN_4: No. 181; N_3NN_1: Nos. 65, 80, 94; N_4NN_2: Nos. 142, 153; N_4NN_4: Nos. 16, 21, 54, 57, 146, 152, 154, 156; N_0NN_2: No. 133; N_0NN_4: No. 74; N_3NN_0: No. 169; N_4NN_0: No. 135; total, 25 pairs.

Harnly ('26, page 321) reported on 32 eggs that were cut into equal fragments along a plane perpendicular to a line connecting the nucleus with the center of the egg, the nucleus being near the cortex at the time of section of the egg. Of these 32 eggs the fourth cleavage took place in both halves of 19 eggs. He found that "in every one of these the nucleated fragment divided into the usual number of mesomeres, macromeres and micromeres at the fourth cleavage, but the non-nucleated fragments divided into two tiers of eight cells." It was this decisive result that enabled him to reach his conclusion that in *Arbacia* "it may appear that localization has taken place in the cytoplasm after maturation and prior to fertilization in such a way that the micromere-forming material lies in the region between the pronucleus and the center of the egg."

In our work on *Lytechinus*, in 30 pairs of fragments only one pair (No. 35) followed the segmentation pattern given by every pair, without exception, of Harnly's 19 pairs from *Arbacia* eggs.

In order to find whether the conflicting results were due to different material, two of us, following the conclusion of the work at Tortugas, worked on *Arbacia* eggs at Wood's Hole during the latter part of August. We could not confirm Harnly's results, but instead found that the fragments of *Arbacia* eggs followed the same cleavage patterns as those exhibited by *Lytechinus*. (Further mention of this study of *Arbacia* will be made in a later section of this report.)

It is also of interest to compare results based on observation of the cleavage in fragments of eggs obtained by section in a plane parallel to a line connecting the nucleus with the center of the egg.

Our observations concerning the formation of micromeres in *Lytechinus* were complete for both fragments of 28 eggs that were cut in such a plane. Table 2 gives a summary of these results.

TABLE 2.—*Lytechinus. Section in a plane parallel to the line, etc.*

Fragments	No micromeres	One or more micromeres on both	Two or more on the nucleated and none on the non-nucleated
Nucleated 28.....	2	19	7
Non-nucleated 28.....	2	19	7

Harnly's observations on micromere formation in *Arbacia,* following cutting in this plane with the nucleus lying close to the plane of division, are summarized in his Table 5 ('26, page 323). This table includes the results from 34 eggs.

Harnly's Table (Nucleus close to line of section) on Arbacia.

Fragment	Fourth cleavage normal	Undetermined	Polyspermy	2 micromeres, 2 macromeres	3 micromeres, 3 macromeres
Nucleated 34....	28	3	3	.....	.....
Enucleated 34....	26	2	.....	4	2

EGG FRAGMENTS OF ARBACIA

A study of egg fragments of *Arbacia punctulata* was made at the Marine Biological Laboratory, Woods Hole, Massachusetts.

The work was done in two periods, the first of three days, August 19 to 21, when 24 pairs of fragments were studied; the second of seven days, September 3 to 10, when 45 pairs were studied. Possibly because of the lateness of the season the material was in poor condition for experimental work.

During the first period, eggs in which the nucleus was close to the surface were cut into two fragments that were as nearly equal in size as possible, the plane of section being at right angles to a line connecting the nucleus with the center of the egg. This orientation was similar to that used by Harnly (1926) when he reached the conclusion that "in all cases in which the nucleated half divided normally through the third cleavage, the pattern of the fourth was like that of the whole egg, and the non-nucleated half segmented at the fourth cleavage into two tiers of eight approximately equal blastomeres."

From our 24 pairs of fragments 3 pairs were obtained in which both

members of the pair went through the first four cleavages regularly, four micromeres being formed; one pair in which there was an equal division of cells to the 16-cell stage, without the formation of micromeres; one pair in which the failure to divide of one prospective macromere gave a 15-cell stage with 3 micromeres for the nucleated fragment, while the non-nucleated fragment divided to 16 cells without the formation of micromeres; one pair in which the nucleated fragment cleaved regularly, while the non-nucleated fragment, as a result of failure in development of one of the blastomeres of the 2-cell stage, gave a typical half cleavage to 2 and 4 cells (equivalent to 4 and 8 had both blastomeres divided), 2 of the 4 cells forming micromeres; and one pair in which only the nucleated fragment developed. Of the remaining 17 pairs, 3 did not become fertilized, 10 were rejected because of irregularity of cleavage, usually preceded by the formation of protrusions; one developed too far for its cleavage to be studied; and our record of three is incomplete.

The results of the study of the pairs that developed successfully in the first period are shown in table 3.

TABLE 3—*Arbacia eggs of successful development in the first period. All cuts were made at right angles to line connecting nucleus with center of the egg. The nucleus was at the greatest possible distance from the cut surface.*

Date	Nucleated fragment	Micromeres	Non-nucleated fragment	Micromeres
8/19	No. 7 Cleavage to 12 cells.....	4	Cleavage to 12 cells.....	4
8/20	No. 6 Cleavage to 16 cells.....	4	Cleavage to 16 cells.....	4
8/20	No. 7 Cleavage to 15 cells.....	3	Cleavage to 16 cells.....	0
8/20	No. 8 Cleavage to 16 cells.....	4	Cleavage to 16 cells.....	4
8/20	No. 10 Cleavage to 16 cells.....	4	Did not develop.....	.....
8/20	No. 11 Cleavage to 16 cells.....	0	Cleavage to 16 cells.....	0
8/21	No. 2 Cleavage to 16 cells.....	4	Cleavage to 7 cells.....	2

Examination of the table will show that 3 of the 6 pairs had 4 micromeres on both fragments; one pair had no micromeres on either; one pair had 3 micromeres on the nucleated fragment and none on the non-nucleated; one pair had 4 micromeres on the nucleated fragment, while the non-nucleated fragment, only half of which cleaved, had 2 micromeres.

The results indicated that there is not a complete localization of micromere-forming substance in the region between the nucleus and the center of the egg.

In the work of the second period the eggs were oriented by the use of a suspension of Chinese ink in sea-water. With one exception, in which the cut was diagonal, all of the cuts were horizontal. 37 of them were both horizontal and at right angles to a line connecting the nucleus with the center of the egg. 14 of the 45 operations were either fully or partially successful. These cases of successful development in the second period are shown in table 4.

TABLE 4.—*Arbacia eggs of successful development in the second period.*

Date	Case		Nucleated fragment		Non-nucleated fragment	
			Total No. of blastomeres	Micro-meres	Total No. of blastomeres	Micro-meres
	No.					
9/3	3		12	4	Undetermined	
9/3	4		16	0	16	4
9/4	1		16	0	Endoplasmic bud	
9/4	5		16	0	16	0
9/6	6		12	4	9	1
9/8	1		12	0	16	2
9/8	3		16	4	Cleavage irregular	
9/8	5		16	0	No development	
9/9	1		16	2	16	0
9/9	2		Cleavage irregular		16	4
9/9	4		12	3	Cleavage irregular	
9/10	1		Not inseminated		16	4
9/10	2		12	0	16	0
9/10	3		12	4	12	4

The evidence from 6 pairs was complete. In 2 of these pairs neither the nucleated nor the non-nucleated fragment formed micromeres, the pattern of the fourth cleavage being that given by the equal division of each cell of the 8-cell stage. In one pair both fragments formed 4 micromeres, in one pair the nucleated fragment formed no micromeres and the non-nucleated formed 2, in one pair the nucleated fragment formed no micromeres and the non-nucleated formed 4, and in one pair the nucleated fragment formed 2 micromeres and the non-nucleated formed none. A complete record of this investigation is given in the Appendix.

OBSERVATION OF MICROMERE FORMATION IN WHOLE EGGS

After the completion of the work from No. 1 to No. 270, inclusive, one of us made a few tests for the purpose of determining whether the variability in cleavage that had been observed in fragments was due to methods of handling the material. It was decided to inseminate whole eggs, giving them precisely the same treatment that had been given the fragments. Single eggs were therefore picked up with a lip pipette and transferred to Bureau of Plant Industry glasses containing 1 c.c. of sea-water. They were then inseminated with 1 drop of the standard dilute sperm suspension. For detailed examination the egg was transferred with a lip pipette to a slide and covered with a supported cover-slip.

Some of these eggs followed the normal cleavage pattern, others showed variations in number of micromeres similar to those that were observed on the egg fragments, while still others either failed to become fertilized or cleaved abnormally.

The observations were so surprising, the results being in many ways so similar to those obtained with fragments and so unlike those expected in the cleavage of whole eggs, that it seemed desirable to make the same kind of study on eggs handled with a lip pipette, but allowed to develop in a larger quantity of water. An examination of single eggs isolated and inseminated in a Syracuse watch-glass containing 15 c.c. of sea-water was therefore made. The manner of development of these eggs was essentially the same as that of the eggs that were isolated in the smaller watch-glasses.

The observations were then extended to eggs from mass inseminations made in 500 c.c. of sea-water, and it was found that normal cleavages were more frequent in these than in the eggs that had been isolated in the smaller dishes.

The results of the three methods of procedure are summarized in the following table. Eggs that followed the regular 2-, 4-, 8- and 12-cell cleavage, giving 4 micromeres, 4 mesomeres and 4 macromeres are classed as "typical"; those that showed variation in the number of micromeres, associated with the equal division of prospective macromeres, are classed as "atypical." The details of these observations are given in the Appendix.

1926

July 23

12 eggs, each isolated and inseminated in 1 c.c. sea-water.
4 did not form a fertilization membrane; did not cleave.
8 developed { 1 not determined; 3 typical; 4 atypical } 66 per cent developed.

July 24

12 eggs, each isolated and inseminated in 1 c.c. sea-water.
1 did not form a fertilization membrane; did not cleave.
1 formed a poor fertilization membrane; did not cleave.
10 developed { 1 tetraster; 1 cytolized in 8-cell stage; 6 typical (2 became atypical later); 2 atypical } 83 per cent developed.

July 25

17 eggs, each isolated and inseminated in 15 c.c. sea-water.
5 did not form a fertilization membrane; did not cleave.
12 developed { 7 typical; 3 atypical; 1 lost; 1 irregular cleavage } 70 per cent developed.

July 28

15 eggs taken in a chance sample from a mass insemination in 500 c.c. sea-water.
15 developed { 12 typical; 3 atypical } 100 per cent developed.

July 30

12 eggs taken in a chance sample from a mass insemination in 500 c.c. sea-water.
12 developed { 10 typical; 2 atypical } 100 per cent developed.

An important fact established by these observations is that entire eggs, either isolated or in mass cultures, may show the same varieties of atypical micromere formation that are shown by egg fragments. It is therefore evident that observations on the number of micromeres formed by a given fragment can have no value in indicating the potential mesenchyme value of the fragment. This confirms the conclusion already drawn from the analysis of the data on developing fragments.

This point being established, we may turn to the consideration of the influence on result of other factors in the method.

The actual cutting of the egg can not be regarded as the only probable cause of inhibition of the formation of micromeres by fragments; this is shown by the observation that entire eggs, like fragments, may form no micromeres. Further, a distinction should be made between slight modifications in development and failure in development. It is conceivable that in some instances the injury to the egg may be so great that development must fail. From this study of the cleavage of isolated whole eggs it seems probable that at least 30 per cent of the failures in development of fragments should not be charged against the actual cutting.

It is clear that the chances for the development of an egg in one of the

mass cultures were greater than those of an isolated egg. This is not because of the amount of water per egg in the culture. In each mass culture there were at least a thousand eggs, about one egg to each 0.5 c.c. of sea-water, which eliminates the idea of failure in development of the single eggs in the 1 c.c. dishes as being due to production of CO_2 by the egg and changed pH. This conclusion is confirmed by the fact that the chances for development of single eggs isolated in 15 c.c. of sea-water were little, if any, better than those of the eggs isolated in 1 c.c. of sea-water.

The eggs that were isolated were transferred with a lip pipette whose bore was small, but not small enough to compress or elongate the egg as it was drawn into the pipette. Furthermore the eggs were not drawn into the pipette violently, but were taken up and expelled with minimum agitation. Nevertheless, the possibility that the process of transferring individual eggs with the pipette has an effect on the eggs, that may induce atypical cleavage, is not excluded.

Probably, however, the rise in temperature which is more rapid in the small dishes containing 1 c.c. or 15 c.c. of sea-water than in the larger dishes containing 500 c.c., is the most disturbing factor in the method that was used. In the temperatures that prevail at Tortugas, the summer temperatures of the sub-tropics, an increase of from 2° to 2.5° C. in the temperature of the sea-water in the small dishes during the 3 hours that their contents were kept under observation, was not uncommon. The range may be from 28.5° to 30.5°, or from 29.5° to 32°, as has been noted in the detailed description given in the Appendix. This maximum temperature is very close to the upper limit of the range in which the normal development of these eggs is possible.

Various methods were used in an attempt to keep the temperature down while the eggs in the dishes were under continuous observation. None of them was satisfactory. Lack of adaptable apparatus prevented a serious attempt at temperature control.

CONSIDERATION OF EVIDENCE OF LOCALIZATION OF ENDODERM-FORMING MATERIAL

If our investigation had included nothing further than the study of formation of micromeres we should have been led to the conclusion that the unfertilized egg of *Lytechinus* is *isotropic*. In support of that idea would have been the fact that fragments (spherical segments) from any part of the egg may form micromeres, and by the fact that in fragments a new polarity may be established. But the investigation did not stop with the study of formation of micromeres. It also included the study of other blastomeres and of the development of fragments taken from different parts of the surface of the egg. These studies showed that there is some degree of separation of ectoderm-forming and endoderm-forming substance in the egg.

Our conclusion then is that the unfertilized egg of *Lytechinus* is neither isotropic nor is it in a state of complete prelocalization such as has been inferred for *Paracentrotus*. Our studies have shown that the unfertilized egg of *Lytechinus* has passed beyond the isotropic condition and that ectoderm-forming and endoderm-forming materials have been partially separated (differentiated) in the egg. In other words a segregation in the egg of materials destined to form particular tissues or organs has taken place.

We conceive of differentiation as resulting in prelocalization. The unfertilized eggs of *Lytechinus* with which we worked showed incomplete prelocalization of ectoderm- and endoderm-forming materials.

The first suggestion of the persistence of an established organization came from the study of No. 223. The plane of section was horizontal. The animal fragment contained the nucleus and was about half as large as the non-nucleated vegetal fragment, that is, it had slightly less than one-third the volume of the entire egg.

The nucleated fragment divided to 4 cells by 2 regular divisions, and to 8 cells by a third division. The 8-cell stage consisted of 4 larger cells of equal size and 4 smaller cells, three of which were of the same size, while one was larger (fig. 28).

In the next division 3 of the small cells each formed a micromere and one divided equally. The 4 larger cells divided equally. The result was a 16-cell stage in which the 8 "mesomeres" were the largest cells, while the 4 "macromeres" were distinctly smaller than the "mesomeres." On the following day this fragment had developed into a gastrula with a small archenteron and with thickened and shortened skeletal rods.

The cleavage in this fragment was neither like that of the whole egg nor like that of the usual fragment. It was instead like that of a fragment in which the position of the planes of cleavage of the entire egg had not been affected by the removal of a part of the egg. At the conclusion of the third cleavage the fragment looked approximately like a truncated model of the normal 8-cell stage of an entire egg.

Fig. 28

Fig. 29

Fig. 30

The striking feature of the cleavage of the fragment was the formation of micromeres by the smaller cells of the 8-cell stage, cells which could be recognized as equivalent to the usual lower cells by their behavior alone, and not, as is usual, by their larger size.

The possible significance of this cleavage having become evident, an examination of our records was made to find whether there had been

similar cases, and an attempt to duplicate the results with new material was begun.

Our records showed only one case, No. 203, in which the first three cleavages were somewhat similar to those of No. 223, and here any further confirmation was lacking as no micromeres were formed. Our efforts to obtain new evidence met with no success until we divided the egg in such a way that the animal fragment was the larger. From this material we obtained ten fragments, a detailed description of which is necessary.

Fig. 31

Fig. 32

No. 235. Plane of section horizontal. The first two cleavages of the nucleated fragment were regular. The third cleavage resulted in an 8-cell stage in which the 4 prospective mesomeres were larger than the prospective macromeres. Three of the smaller cells each formed a micromere; one did not divide. The first two cleavages of the non-nucleated fragment were regular. The third cleavage was confined to 2 of the cells, resulting in a 6-cell stage. In the next division the 4 upper cells divided equally; 1 of the 2 lower cells formed a micromere, the other divided equally, giving a 12-cell stage.

No. 238. Plane of section horizontal. The first two cleavages of the nucleated fragment were regular. The third cleavage was similar to that shown in figure 28 and formed an 8-cell stage of 4 large and 4 small cells. Each of the 4 small cells produced a micromere, one of the micromeres being larger than any of the other 3. The 4 large cells divided equally one minute after the completion of the division of the small cells. The first cleavage of the non-nucleated fragment was regular; the second was completed in only one of the blastomeres.

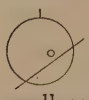
No. 242 (Fig. 29). Plane of section supposed to be horizontal, but the orientation was not thoroughly dependable. The first cleavage of the nucleated piece was regular; the second was parallel to the cut surface (fig. 30); the third at right angles to the cut surface, giving 8 cells, 4 of which were large and 4 small (fig. 31). Two of the large and 2 of the small cells formed micromeres. The first 4 cleavages of the non-nucleated fragment were regular, giving 4 micromeres, 4 macromeres and 8 mesomeres. The mesomeres were very small, smaller than the micromeres.

No. 252 (Fig. 32). Plane of section horizontal. The first two divisions in the nucleated fragment were regular; the third gave 4 cells that were slightly smaller than the other 4; 2 of the smaller cells formed micromeres; the other 2 divided equally. The first three cleavages of the small non-

nucleated fragment were regular, resulting in 8 cells, 4 of which were larger than the other 4. The 4 larger divided slightly unequally, one minute before the smaller divided equally.

No. 254. Plane of section horizontal. The first 3 divisions in the nucleated fragment were regular. In the next division the fragment passed from the 8 to the 16-cell stage, 4 cells dividing slightly earlier than the other 4, division in all being equal. No. 254 differs from any of the cases presented thus far in that the large animal fragment is the non-nucleated piece. The first 3 divisions in this non-nucleated animal fragment were regular, resulting in 4 larger and 4 smaller cells. 3 of the smaller cells each produced a micromere, the fourth divided equally. The 4 larger cells divided equally one minute after the smaller cells had formed micromeres, each of the 8 mesomeres being of the same size as the "macromeres."

No. 280. Plane of section diagonal. The first 3 divisions in the nucleated fragment resulted in an 8-cell stage in which there were 4 large and 4 small cells. Each of the small cells formed a micromere. In the 16-cell stage each of the 4 "macromeres" was of about the same size as each of the 8 mesomeres. The non-nucleated fragment did not develop.



No. 281. Plane of section diagonal. The nucleated fragment did not develop. The first 3 divisions in the non-nucleated fragment resulted in an 8-cell stage in which there were 4 large and 4 small cells. 3 of the 4 large cells produced micromeres and one divided equally. Two minutes later the 4 small cells divided to form 8 mesomeres, each of which was only slightly larger than the micromeres. In this instance it seems possible that the greater part of the ectoderm-forming material had been cut off in the nucleated fragment, while the non-nucleated fragment contained only a small amount of this material, represented by the 8 strikingly small mesomeres.

No. 282. Plane of section diagonal. Animal fragment non-nucleated. The first 3 divisions in the nucleated fragment resulted in 8 cells, 4 of which were smaller than the other 4. The 4 small cells divided unequally; the large cells divided equally. The first 2 cleavages of the non-nucleated (the animal) fragment gave an irregular 4-cell stage, this later became regular.

No. 283. Plane of section diagonal. The first 3 divisions in the nucleated fragment resulted in 8 cells, 4 of which were smaller than the other 4. 2 of the smaller cells formed micromeres, while 2 divided equally. One minute after the division of the smaller cells, the larger cells divided equally. The cleavage of the non-nucleated fragment was not followed after the 2-cell stage.

No. 285. Plane of section diagonal. Attention is again directed to the fact that in this case as in Nos. 254 and 282 it is the large animal fragment that is non-nucleated. The first 3 divisions in the nucleated fragment produced 8 cells of nearly equal size. In the next divisions 3 cells each produced a micromere, while one cell divided equally. The upper 4 cells divided equally to form 8 mesomeres. The first 3 divisions in the non-nucleated fragment produced 8 cells, 6 of which were large and 2 very small. 2 of the large cells divided equally while the 2 small cells were forming micromeres. The remaining 4 cells divided equally to form 8 mesomeres.

It is important that the nature of the fragments under consideration be kept in mind. We therefore repeat their description in the form of a table.

No. of Egg	*Description of fragment*	*Plane of section*
223	Nucleated animal, less than half the egg.	Horizontal
235	Nucleated animal, more than half the egg.	Horizontal
238	Nucleated animal, more than half the egg.	Horizontal
252	Nucleated animal, more than half the egg.	Horizontal
280	Nucleated animal, more than half the egg.	Diagonal
283	Nucleated animal, more than half the egg.	Diagonal
254	Non-nucleated animal, more than half the egg.	Horizontal
285	Non-nucleated animal, more than half the egg.	Diagonal
242	Non-nucleated vegetal, less than half the egg.	Horizontal
281	Non-nucleated vegetal, less than half the egg.	Diagonal
282	Nucleated vegetal, less than half the egg.	Diagonal

In all of the animal fragments (8 cases), the 8-cell stage consisted of 4 larger and 4 smaller cells and the micromeres were formed by the smaller cells. The form of the cleavage suggests that there was prelocalization of prospective endoderm in the egg and that the first horizontal cleavage was approximately where it might have been in the whole egg. This may be more readily comprehended when it is remembered that in animal fragments obtained by horizontal section the direction of the planes of cleavage is the same as in the entire egg, and that in animal fragments obtained by diagonal section the direction of the planes of cleavage is shifted through less than 45 degrees.

The three vegetal fragments, Nos. 242, 281 and 282, all fragments smaller than half the egg, furnish evidence complementary to that presented above. In these instances it is prospective ectoderm that was removed. In their cleavage all of these fragments formed small mesomeres, in one instance (No. 242) smaller than the micromeres; in the other (No. 281) only slightly larger than the micromeres.

Egg No. 271 not included in the preceding discussion, also presents evidence in support of the idea of prelocalization. The first three divisions in the nucleated fragment were regular and resulted in an 8-cell stage in which there were 4 smaller and 4 larger cells. The 4 smaller cells formed micromeres, 2 of the micromeres having twice the

diameter of the other 2; the 4 larger cells divided equally. In this fragment the micromeres were not formed on the cut surface, but at the intersection of the first two planes of cleavage opposite the surface of section. Polarity was swerved through about 40 degrees (fig. 33). Our only comment on the fact that 2 of the micromeres were large and 2 small is that it is suggestive, but because of the variability in size and number of micromeres that has been evident throughout our work, it can not be regarded as conclusive evidence of prelocalization.

Fig. 33

The facts already presented concerning the fragments of Nos. 256, 257, 260, 261 and 262 (page 22), all obtained by vertical section in which the first cleavage amphiaster appeared in the short axis of the fragment, also suggest a tendency toward the persistence of an established organization.

Finally, the most conclusive evidence that there is a certain amount of prelocalization of ectoderm-forming and endoderm-mesenchyme-forming material in the unfertilized egg is that furnished by the blastulæ and gastrulæ derived from fragments taken as small segments of different regions of the egg. As examples, the evidence afforded by two contrasting cases, Nos. 275 and 283, will be presented.

In No. 275 the small, non-nucleated animal fragment, containing only part of the material of the animal half of the egg, developed into a blastula with 4 mesenchyme cells, while the large nucleated fragment, containing all of the material of the vegetative half and part of the material of the animal half of the egg, developed into a gastrula with mesenchyme, skeletal spicules and pigment.

In No. 283, page 44, in which the plane of section was near the vegetal pole, the small segment developed into a very compact gastrula in which there was a solid looking archenteron closely surrounded by mesenchyme cells, while the larger fragment gave rise to a normal gastrula with mesenchyme and skeletal spicules.

The impression given by these two gastrulæ was that one had an insufficient amount of ectodermal covering in proportion to its endoderm and mesenchyme, while the other had more than the usual proportionate amount of ectoderm. Similar evidence is given by the development of the fragments of eggs Nos. 253, 276, 278 and 279.

In summarizing it may be said that support to the idea of prelocalization of endoderm-forming material in the unfertilized egg is given by:

(1) The character of cleavage in fragments which contain all of the material of the animal hemisphere, plus some of the material of the vegetative hemisphere, *i.e.*, large fragments which have been obtained by cutting the egg in a plane below that of the equator. In fragments of this kind

the cells which produce the micromeres are smaller than those which form the mesomeres.*

(2) The ability of small segments taken from the region of the animal pole to develop only to the blastula stage, as contrasted with the ability of small segments from the region of the vegetal pole to develop to gastrulæ and later stages.

Having presented the evidence in support of the idea of prelocalization, we turn naturally to the consideration of that against the idea, and discover the rather surprising fact that there is little evidence to present.

During the course of our work in 1926, 20 eggs, of which one or both fragments were fertilized and developed, were cut in a plane which gave a large animal and a small vegetal fragment. The remaining 62 eggs, of the total of 82 cut horizontally or diagonally which might have furnished material of this kind, were cut either into equal fragments or to fragments of which the animal segment was the smaller.

10 of these 20 eggs, namely Nos. 235, 238, 242, 252, 254, 280, 281, 282, 283 and 285 have been described as showing evidence of prelocalization. One, No. 251, formed an 8-cell stage in which there were 4 large and 4 small cells; but at the next division all of these cells divided simultaneously and equally. Six, namely, Nos. 243, 244, 245, 248, 249 and 250, all cut within a period of an hour, were fertilized and developed, but by the time that the studies of Nos. 242 and 247 was completed, had developed beyond the early cleavage stages.

There thus remain three eggs, Nos. 247, 253 and 284, from which there is a possibility of obtaining evidence against prelocalization.

The smallest possible nucleated fragment was cut from the vegetal pole of No. 247. The large fragment remaining, containing about 36/37 of the egg, formed an 8-cell stage in which the cells were of equal size. 4 micromeres were formed at the next division. The large animal fragment from No. 253 consisted, at the 8-cell stage, of 6 larger and 2 smaller cells. 2 of the larger cells formed large micromeres and the 2 small cells formed small micromeres. The large animal fragment of No. 284 formed 8 mesomeres, while 2 of the lower cells formed micromeres and 2 divided equally.

None of these eggs can be said to furnish evidence against prelocalization. When it is remembered that in the entire egg the 4 micromere-producing cells of the 8-cell stage are usually larger than the 4 mesomere-producing cells it is apparent that No. 247 gives as much evidence for localization as against it. The same thing may be said concerning No. 253. There still remains No. 284. The fact that 2 of the antipolar cells formed micromeres while 2 divided equally does not detract from the value of this fragment as evidence, for this deviation from the normal occurs even in whole eggs.

* One of us, Dr. Taylor, doubts the significance of cell-size as indicative of a segregation of endoderm-forming material in the unfertilized egg.

But, even giving No. 284 full value as evidence, all that can be said concerning it is that it showed no indication of differentiated endoderm-forming substance. In final analysis then:

(1) Out of 14 eggs that were cut so as to give a large fragment containing more than the animal half of the egg, and a small fragment containing less than the vegetal half, one or both fragments of 11 eggs cleaved in a manner that indicated prelocalization of endoderm-forming substance; 2 can be interpreted as showing evidence of prelocalization, although taken by themselves they would not suggest it; and one gives no evidence of prelocalization.

(2) The fact that in certain fragments obtained by vertical section the first cleavage amphiaster lies temporarily in the short axis of the cell, suggests the persistence of an established organization.

(3) The fact that small animal fragments develop only to the blastula stage, while small vegetal fragments may develop to gastrulæ and later stages, seems to give conclusive evidence that, in certain cases at least, there has been differentiation and localization of endoderm-forming material.

POLARITY AND SYMMETRY IN THE SEA-URCHIN EGG

Wilson ('95) showed that in *Lytechinus* the spermatozoön may enter the egg at any point and that the plane of the first cleavage is determined by the poles and the point of entrance of the spermatozoön, the spermatozoön thus fixing the meridian bounding the first cleavage plane.

Boveri ('02 and '05) believed that in *Paracentrotus* the usual point of entrance is at the micropyle, thus at the animal pole. Boveri held that the first cleavage plane is in the median body plane. Runnström ('14 and '19) obtained results that confirmed Boveri's conclusion. Driesch ('06) decided that the first cleavage plane is at right angles to the future median plane of the larva, and ('08) that the first plane separates dorsal from ventral.

Von Ubisch's ('25) work is conclusive in its evidence that the first cleavage plane stands in no constant relation to the median plane of the larva. He showed that it coincided with the median plane in 19 out of 38 cases in *Echinus esculentus;* that it was in the future horizontal plane of the larva, separating dorsal from ventral in 13, oblique in 4, and not clear in 2 cases. Results of the same character were obtained with *Echinus miliaris* and *Echinocyamus pusillus.*

There is, therefore, no evidence that bilaterality is indicated by the position of the first cleavage amphiaster in the egg, which might be the case if the plane of the first cleavage invariably separated right from left, or dorsal from ventral. So far as evidence shows, the blastula first becomes bilaterally symmetrical when two mesenchyme groups are formed.

Our own observations that in *Lytechinus* the polar bodies are given off at the base of the funnel and that the first two cleavage planes cross each other at right angles at the animal and vegetal poles confirm those of other observers.

As the anterior end of the larva always coincides with the animal pole of the egg, and as the micromeres—and in turn the mesenchyme cells—are always formed at the vegetal pole, it seems to be a fact that the egg has polarity.

Polarity is basic. It is the fundamental feature of organization. The differentiation of derm-forming stuffs is secondary and incidental.

Historically, with the establishment of polarity the major axis of the embryo is determined. What is the next step? Does it lie in the direction of derm-forming stuffs or is there some more general and fundamental feature of organiaztion?

The fact of organization with respect to polarity has been shown to exist in the *Lytechinus* egg by (1) the tendency of the first cleavage amphiaster to appear in the short axis of fragments obtained by vertical section (pages 22-23); (2) localization of endoderm-forming material (pages 41-45); (3) localization of ectoderm-forming material (pages 45-46). It has also been shown that this organization is modifiable, even to the extent of changing the polarity (page 21).

Do these recognizable features of organization precede the establishment of symmetry? It is conceivable that symmetry is a fundamental attribute of organized material, secondary only to polarity, and that the establishment of minor axes incident to bilaterality is the first step in the epigenetic development of the larva, taking precedence historically over the processes of differentiation of derm-forming stuffs.

It may be urged that as radially symmetrical animals have germ layers, bilaterality is a particular and not a general feature of development, and that the establishment of bilaterality is one of the later attributes of organization.

It is commonly assumed that the sea-urchin egg is radially symmetrical about the egg axis. Is this a fact? When is bilaterality established? Is it induced from the outside or does it exist in the unfertilized egg? What determines the bilaterality of the parthenogenetic larva?

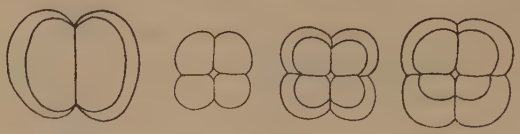
The significance of these questions makes inquiry desirable.

DIAGONAL FRAGMENTATION AND BILATERALITY

Certain size relationships in the cells of fragments that had been obtained by diagonal cuts suggested a consideration of this asymmetry in its possible connection with bilaterality.

Seventeen eggs, from 14 of which fragments developed successfully, were cut diagonally. Some of these eggs showed an asymmetry of cleavage which it was thought might have some connection with localization, specifically, that in the 4-cell stage a given cell might represent a right or left ventral or a right or left dorsal segment, a distinction which would become more evident when the egg passed into the 8-cell stage and an anterior and a posterior group of cells was established.

For the purposes of this consideration cleavage in which there is evident variation in size among either the 4 cells of the animal half or the 4 cells of the vegetative half of the egg will be regarded as asymmetrical (figs. 34,



34 35 36 37

35, 36, 37). Variation in size among *micromeres* can not serve as a criterion of *asymmetry*, for the reason that it has been evident throughout our studies that the 4 cells of the vegetal half of the egg have no constancy in manner of division. The essential facts will be presented as briefly as possible:

No. 271. Nucleated fragments, 4 of cells smaller; 4 larger (fig. 33). 4 smaller formed micromeres, 2 of which were twice the diameter of the other 2. The 2 larger micromeres were those situated nearer the vegetative pole of the egg. Polarity was shifted through about 40°. Conclusion: Cleavage symmetrical, no differences in size between the cells of each group of 4 of the 8-cell stage being evident. Non-nucleated fragment did not develop.

No. 272. Nucleated fragment, went to 8-cell stage, then through cell fusion went back to 6 cells; 1 of the 6 formed a micromere; 1 divided slightly unequally, 4 divided equally, giving a 12-cell stage. Conclusion: Evidence inconclusive. Non-nucleated fragment cleavage irregular.

No. 273. Nucleated fragment, went to 8 cells; 2 micromeres formed; 2 of the vegetal 4 did not divide; 1 of these was large, 1 small, agreeing in form with the original shape of the fragment, which had rounded up, however, before division. Conclusion: Cleavage asymmetrical. Non-nucleated fragment did not develop.

No. 275. Nucleated fragment, in 8-cell stage there were 4 cells of equal size and a group of 4, of which 2 were larger and 2 smaller. The 4 cells of equal size each gave rise to a micromere. Each of the other 4 divided equally, giving 4 mesomeres that were smaller than the other 4. Conclusion: Cleavage asymmetrical. Non-nucleated fragment; cleavage equal, to 16 cells; no micromeres.

No. 276. Nucleated fragment, cleavage symmetrical, the only unusual feature being a delayed formation of the mesomeres. The 16-cell stage consisted of 2 rows of 4 cells, between which was a row of 8 large cells. Non-nucleated fragment, symmetrical 4-cell stage.

No. 277. Nucleated fragment, 12-cell stage with 4 micromeres, 2 of which were large and 2 small. No record of sizes of cells in 4 or 8-cell stage. Non-nucleated fragment, 4 mesomeres, 4 mega-

meres, 5 micromeres. The fifth micromere was formed by a second division of one of the macromeres.

No. 278. Nucleated fragment, regular 8-cell stage; all of the cells divided equally giving a 16-cell stage. Non-nucleated fragment; 4-cell stage of 2 larger and 2 smaller cells; then to 7-cell stage, 1 of the larger cells failing to divide; this large cell then divided unequally; 3 cells then formed micromeres, and the remaining 3 divided equally to form 6 mesomeres, giving a 14-cell stage. Conclusion: Cleavage asymmetrical.

No. 279. Nucleated fragment, regular division to the 8-cell stage; 3 cells formed micromeres, all others divided equally giving a 16-cell stage. Conclusion: Cleavage symmetrical. Non-nucleated fragment, regular division to the 8-cell stage; all cells then divided equally.

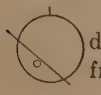
No. 280. Nucleated fragment, in 4 cell stage (fig. 35), 2 cells distinctly smaller; in 8-cell stage (fig. 36) 4 small cells and 4 large; each of the small cells formed a micromere; the large cells divided equally to form 8 mesomeres. Each macromere was of about the same size as each of the mesomeres. Conclusion: Cleavage asymmetrical. Non-nucleated fragment, no development.

No. 281. Nucleated fragment, no development. Non-nucleated fragment in 8-cell stage, 4 cells large, 4 small; one of the large cells divided equally, while 3 formed micromeres; the 4 small cells divided to 8 mesomeres, which were only slightly larger than the micromeres. Conclusion: Cleavage symmetrical.

No. 282. Nucleated fragment, regular 4-cell stage; 8-cell stage, 4 cells smaller than other 4. The 4 smaller cells divided unequally; the 4 larger divided equally. Conclusion: Cleavage symmetrical. Non-nucleated fragment, the cells of the 4-cell stage were at first of irregular form, then rounded up and gave a regular 4-cell stage; one of these cells was slow in its next division, giving a 7-cell stage. Ultimately this passed into a 16-cell stage without micromeres. Conclusion: Cleavage symmetrical.

No. 283. Nucleated fragment, regular 4-cell stage; 8-cell stage, 4 cells smaller than other 4; 2 of small cells formed micromeres, 2 divided equally. The 4 large cells divided equally. Conclusion: Cleavage symmetrical. Non-nucleated fragment, cleavage not followed.

No. 284. Nucleated fragment, 8-cell stage, no information as to cell size; 2 cells formed micromeres, 2 divided equally; 4 cells divided to 8 mesomeres. Conclusion: Cleavage symmetrical. Non-nucleated fragment, cleavage not followed.



No. 285. Nucleated fragment, 3 small micromeres, 1 lower cell divided equally, 4 cells divided to 8 mesomeres. Non-nucleated fragment, 8 cells (fig. 37), 6 large and 2 very small; 2 small formed

micromeres, 2 of the large divided equally; 4 largest divided to 8 mesomeres. Conclusion: Cleavage asymmetrical.

These cases may be sorted, for purposes of comparison, into two natural groups.

DIAGONAL FRAGMENTS—

(1) Animal fragment larger:
- No. 280 Asymmetrical cleavage.
- 281 No development.
- 282 Symmetrical cleavage.
- 283 Symmetrical cleavage.
- 284 No information as to cell size.
- 285 Asymmetrical cleavage.

(2) Vegetal fragment larger:
- No. 271 Symmetrical cleavage.
- 272 Evidence inconclusive.
- 273 Asymmetrical cleavage.
- 275 Asymmetrical cleavage.
- 276 Symmetrical cleavage.
- 277 No information as to cell size.
- 278 Asymmetrical cleavage.
- 279 Symmetrical cleavage.

No essential difference between the cleavages of the animal and vegetal fragments is evident.

Taking the data as they stand it may be seen that in 10 cases in which the evidence is conclusive, out of the 14 fragments obtained by diagonal cutting that developed, 5 cleaved symmetrically and 5 asymmetrically.

These facts, considered without reference to related observations, have no especial significance. It seems desirable, therefore, to compare the cleavage of the oblique fragments with that of similar fragments obtained by horizontal cutting.

HORIZONTAL FRAGMENTS—

(1) Animal fragment larger:
- No. 192 Symmetrical cleavage.
- 235 Symmetrical cleavage.
- 238 Symmetrical cleavage.
- 247 Symmetrical cleavage.
- 251 Symmetrical cleavage.
- 252 Symmetrical cleavage.
- 253 Symmetrical cleavage.
- 254 Symmetrical cleavage.

(2) Vegetal fragment larger:
- No. 193 Asymmetrical cleavage.
- 199 Symmetrical cleavage.
- 206 Symmetrical cleavage.
- 210 Symmetrical cleavage.
- 214 Symmetrical cleavage.
- 215 Symmetrical cleavage.
- 216 Symmetrical cleavage.
- 217 Symmetrical cleavage.
- 225 Symmetrical cleavage.

(2) Vegetal fragment larger: (Continued)
No. 226 Evidence inconclusive.
227 Symmetrical cleavage.
229 Symmetrical cleavage.
269 Symmetrical cleavage.
274 Symmetrical cleavage.

In our material there are 21 fragments made by horizontal cutting from which conclusions as to symmetry or asymmetry of cleavage may be drawn. 19 of these fragments cleaved symmetrically, 2 cleaved asymmetrically.

A comparison of the results from the two groups, 5 cases of asymmetrical cleavage out of 10 diagonal fragments, against 2 out of 21 horizontal fragments; and 5 cases of symmetrical cleavage out of 10 diagonal fragments against 19 out of 21 horizontal fragments, leads at least to the conclusion that there may have been a difference in nature in the fragments of the two groups. We do not feel, however, that the evidence enables us to say that the persistence of a bilaterally symmetrical organization has been demonstrated. It is clear that following diagonal section, there may be a delayed re-arrangement of material that sometimes results in asymmetrical cleavage. The question should be investigated further.

DISTANCE OF NUCLEUS FROM PLANE OF SECTION AND EXTENT OF DEVELOPMENT

This section of the paper is included as a result of a suggestion made by Professor T. H. Morgan to the effect that we ought to present, in addition to observations on the development of small fragments, full data on the position of the nucleus at the time of section, the point being that it should be shown whether a gastrula ever developed from a non-nucleated fragment if the nucleus was not close to the plane along which the cut was made. Data on fourteen pairs of fragments are available for this consideration.

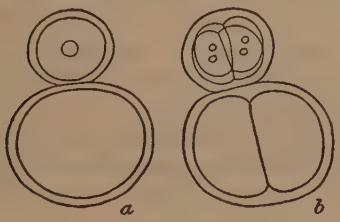


Fig. 38

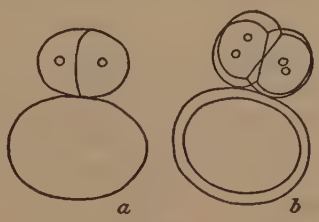


Fig. 39

No. 54 (1924) (Figs. 38 a and b). Horizontal cut. Relative volume 1 to 3.4. Nucleus well within the animal half at time of cutting. Both normal 2, 4 and 8-cell stages; micromeres not determined. Both swimming blastula with mesenchyme 6 hours and 20 minutes after insemination. Nucleated fragment with 4 mesenchyme cells; non-nucleated fragment with 12 mesenchyme cells. Both blastulæ had thick posterior wall. Both developed into plutei.

No. 56. July 23, 1924. (Figs. 39 a and b). Relative volume 1 to 1.7. Nucleus well to one side of the egg axis. Both normal 2, 4 and 8-cell stages; micromeres not determined. July 24, both normal blastulæ and gastrulæ. July 25, both plutei with poorly developed arms, good body skeleton, digestive tracts and pigment.

No. 229. July 17, 1926 (Fig. 40). Relative volume 16 to 1. Nucleus near center of egg. Inseminated 10 h. 27 min., both normal 2, 4 and 8-cell stages. Nucleated fragment 16 cells, 4 micromeres. Non-nucleated frag-

Fig. 40

Fig. 41

Fig. 42

Fig. 43

ment 16 equal cells, no micromeres. 5^h 40^m, nucleated fragment 16 mesenchyme cells. 5^h 40^m, non-nucleated fragment 4 mesenchyme cells. Both normal active larvæ. July 18, 10^h a.m., nucleated fragment; a gastrula with an abnormal amount of mesenchyme. Only one skeletal spicule. Non-nucleated fragment; a fine active blastula with mesenchyme. No evidence of gastrulation. No skeletal spicules. July 19, 9^h a.m., nucleated fragment, a poorly developed pluteus without arms. Had œsóphagus, stomach and intestine. Body skeleton feebly developed but was present on right and left sides of body. Non-nucleated fragment; larva active, one small triradiate spicule present. No evidence of gastrulation.

No. 231. July 17, 1926. (Fig. 41). Relative volume 1 to 1.3. Nucleus in animal half of egg, close to plane of section. Inseminated. Both normal 2, 4 and 8-cell stages. Nucleated fragment followed to 32 cells, no micromeres. Non-nucleated, 2 micromeres; other cells divided equally. 6^h 5^m, nucleated fragment, fine larva, posterior wall thickened, no mesenchyme; non-nucleated fragment, active larva; its blastocœle filled with mesenchyme (?). July 18, 9^h a.m., nucleated fragment, good blastula, no mesenchyme present, simply a ciliated, hollow sphere. Non-nucleated fragment, a gastrula, but cells in blastocœle were too compact to be normal; no skeletal spicules. July 19, 3 skeletal spicules present.

No. 237. July 18, 1926. (Fig. 42). Relative volume 1 to 10. Nucleus well within the animal half. Nucleated 16 cells, no micromeres; non-nucleated, 11 cells, 4 micromeres. July 19, nucleated, dead; non-nucleated, gastrula.

No. 253. July 21, 1926. (Fig. 43). Relative volume 10 to 1. Nucleus close to animal pole. Nucleated, 16 cells, 4 micromeres (2 larger, 2 smaller); non-nucleated, 16 cells, 4 cells not of equal size, but equal division of all cells. July 22, nucleated, excellent normal gastrula; non-nucleated, excellent normal gastrula. July 23, nucleated, pluteus without arms; non-nucleated, disintegrated.

No. 266. July 22, 1926. Relative volume 7 to 1. Nucleus closer to surface of egg than to plane of section. Nucleated, 16 cells—3 micromeres, one cell divided equally (fig. 12); non-nucleated 16 cells, each of 8-cell stage divided equally. July 23, 8.00 a.m., non-nucleated, excellent normal gastrula; very small skeletal spicules, much smaller than normal spicules.

No. 275. August 5, 1926. Relative volume 20 to 1. Nucleus about its own diameter from plane of section. August 6, nucleated, excellent gastrula with mesenchyme, skeletal spicules and pigment; non-nucleated, blastula with 4 mesenchyme cells.

No. 276. August 5, 1926. Relative volume 10 to 1. Nucleus about its own diameter from surface of egg at vegetal pole. August 6, nucleated abnormal gastrula (evaginate archenteron); non-nucleated, blastula with mesenchyme.

No. 277. August 5, 1926. Relative volume 6 to 1. Nucleus nearer to vegetal pole than to plane of section. August 6, nucleated, gastrula with shallow archenteron—too much mesenchyme; non-nucleated, excellent gastrula with skeletal spicules.

No. 278. August 5, 1926. Relative volume 1 to 5. Nucleus less than its own diameter from plane of section. August 6, nucleated, excellent blastula with thickened posterior wall—6 mesenchyme cells in blastocœle (fig. 44); non-nucleated, gastrula, well-developed skeletal rod on one side, spicule on the other (fig. 45).

No. 279. August 6, 1926. Relative volume 10 to 1. Nucleus within vegetal half, somewhat more than its own diameter from plane of section. August 7, nucleated, very good late gastrula or young pluteus. Two large triradiate spicules. Pigmented cells among clear mesenchyme cells and in ectoderm. The outline only is given in figure 46.

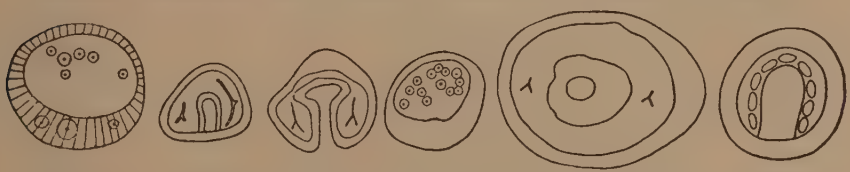


Fig. 44 Fig. 45 Fig. 46 Fig. 47 Fig. 48 Fig. 49

Non-nucleated, excellent blastula with thickened posterior wall, 13 mesenchyme cells (fig. 47).

No. 283. August 6, 1926. Relative volume 5 to 1. Nucleus in animal half about its own diameter from plane of section. August 7, nucleated, gastrula, much mesenchyme, 2 skeletal spicules (fig. 48); non-nucleated, gastrula, small solid archenteron, mesenchyme cells closely packed around archenteron, 2 small tri-radiate spicules (fig. 49).

No. 285. August 6, 1926. Relative volume 1 to 4, nucleus in vegetal fragment close to plane of section. August 7, both partially disintegrated, seem to have gone to gastrula stage.

Nos. 266 and 277, in both of which the nucleus was situated more than three times its own diameter from the plane of section and in both of which the non-nucleated fragment developed to a gastrula, show definitely that it is not necessary that the nucleus be close to the plane of section in order to obtain a gastrula from non-nucleated fragment. Positive proof is shown by No. 253 (fig. 43) in which the nucleus was situated close to the animal pole. The non-nucleated fragment contained the vegetal polar material and was about 1/10 the size of the nucleated fragment. Both fragments formed perfect gastrulæ.

HISTORY OF REARED FRAGMENTS

During the work of 1924 constant effort to rear the fragments to the oldest possible stage was made. The cleaving fragments were isolated in embryological watch-glasses (salt cellars) which were then placed in large moist chambers.

During the studies of 1926 the work of rearing fragments was incidental to the investigation on micromeres, until near the close of that part of the project.

The data on the developmental stages secured have been arranged in tabular form as a short and clear method of presentation.

History of reared fragments.

Operation number	Relative volume nucleated to non-nucleated	Nucleated fragment developed into—	Age in hours	Non-nucleated fragment developed into—	Age in hours
Horizontal sections					
1924 No.					
5	1 : 1	Blastula	21	Ball of small cells	21
6	1 : 1	Blastula	5	Did not segment	.....
8	1 : 1	Pluteus without arms	72	Gastrula (lost)	19
41	1 : 1	Gastrula	24	Failed during segmentation	4
42	1 : 1	Pluteus, normal	30	Gastrula (dead)	20
44	1 : 1	Gastrula	24	Gastrula	24
45	1 : 1	Failed in segmentation	.....	Blastula with mesenchyme	18
47	1 : 1	Pluteus, normal	48	Whirling ball of cells	48
54	1 : 3.4	Pluteus, normal	72	Pluteus, normal	72
57	1 : 1	Did not segment	.....	Gastrula	24
59	1 : 1	Pluteus without arms	48	Did not segment	.....
61	1 : 1	Pluteus without arms	48	Pluteus, normal	48
62	1 : 1	Disintegrated before cleavage	.....	Gastrula	24
66	1 : 1	Lost before insemination	.....	Pluteus, defective	48
68	1 : 1	Pluteus, abnormal	48	Disintegrated during cleavage	2
79	1 : 1	Did not segment	.....	Blastula, abnormal	22
1926					
221	1 : 2	Stereoblastula	21	Stereoblastula	21
223	1 : 2.5	Gastrula	20	Failed during segmentation	2
227	2 : 1	Blastula with mesenchyme	8	Did not segment	.....
229	16 : 1	Pluteus without arms	46	Blastula with mesenchyme	46
230	1 : 1.2	Pluteus without arms	48	Gastrula	22
231	1 : 1.3	Blastula, no mesenchyme	22	Gastrula with spicules	46
237	1 : 10	Disintegrated during cleavage	5	Gastrula	19
252	30 : 1	Gastrula	22	Failed after regular cleavage	4
253	10 : 1	Pluteus without arms	45	Gastrula	22
254	1 : 1.1	Pluteus, left arm lacking	40	Early blastula	5
269	18 : 1	Gastrula	17	Disintegrated in late cleavage	4
Vertical sections					
1924					
17	1 : 1	Blastula	5	Did not segment	.....
23	1 : 1	Blastula	24	Did not fertilize	.....
25	1 : 1	Blastula	22	Irregular mass of cells	22
26	1.5 : 1	Blastula	6	Did not segment	.....
27	1 : 1	Pluteus without arms	96	Did not segment	.....
37	1 : 1	Failed during segmentation	.....	Gastrula, erratic	72
38	1 : 1	Blastula (lost)	6	Gastrula, dead	43
40	1 : 1	Pluteus, normal	36	Pluteus, normal	36
53	1 : 1	Gastrula	24	Gastrula	24
55	1 : 1	Gastrula	22	Blastula with mesenchyme	7
56	1 : 1.7	Pluteus, poor arms	70	Pluteus, poor arms	70
58	1 : 1	Pluteus, poor arms	72	28-cell stage; crushed	.....
60	1 : 1	Blastula with mesenchyme	22	Pluteus, normal	48
67	1 : 1	Fragments fused; blastula; then irregular mass			6
69	1 : 1.5	Lost during cleavage	.....	Gastrula	20
77	1 : 1	Gastrula	20	Injured during cleavage	2
80	1 : 1	Gastrula	20	Gastrula	20
1926					
1	1 : 1	Blastula with mesenchyme	6	Blastula with mesenchyme	6
258	1 : 1	(One disintegrated; one beginning gastrulation)			19
266	7 : 1	Lost during cleavage	.....	Gastrula	18
267	1 : 1	Blastula with mesenchyme	18	Did not segment	.....

History of reared fragments—Continued.

Operation number	Relative volume nucleated to non-nucleated	Nucleated fragment developed into—	Age in hours	Non-nucleated fragment developed into—	Age in hours
Diagonal sections					
1924					
275	20 : 1	Gastrula	16	Blastula, 4 mesenchyme cells	16
276	10 : 1	Gastrula, evaginate	16	Blastula with mesenchyme	16
277	6 : 1	Gastrula	15	Gastrula	15
278	1 : 5	Blastula, 6 mesenchyme cells	22	Gastrula	22
279	10 : 1	Gastrula	21	Blastula with mesenchyme	21
283	5 : 1	Gastrula	17	Gastrula	17
284	44 : 1	Gastrula	17	Did not fertilize	.....
285	1 : 4	Gastrula? (disintegrated)	17	Gastrula? (disintegrated)	17
Random sections					
1924					
12	1 : 1	Blastula	20	Spherical mass of cells	20
16	1 : 2	Blastula	5	Ball of irregular cells	5
20	1 : 1	Pluteus without arms	120	Lost after normal segmentation.	3
21	1 : 1	Gastrula, normal	43	Gastrula, evaginate	43
Cut at right angles to line connecting nucleus with center of the egg					
130	1 : 1	Blastula	8	Blastula	8
133	1 : 1	Blastula with mesenchyme	7	Blastula with mesenchyme	7
134	1 : 1	Blastula	7	Blastula	7
135	1 : 1.2	Blastula	7	Blastula	7

Explanation of Descriptive Terms Used in the Table

Pluteus without arms.—These were plutei that were normal in every respect, with the exception that the post-oral arms were developed only as mere protuberances. The body skeleton was strongly developed and well formed. The basal part of the skeletal rod of the arm was present as a very short projection.

Erratic gastrula.—The term is descriptive of the path followed during locomotion.

Pluteus, defective.—The pluteus from the non-nucleated fragment of No. 66 was defective in that it had a single median "post-oral" arm containing two skeletal rods; this single arm therefore represented the two post-oral arms of the normal pluteus.

Failed after regular cleavage.—This fragment (No. 252) cleaved normally, forming micromeres, to a fine 16-cell stage. It was transferred for rearing, but when examined on the following morning was dead.

The following additional information, that can not be placed in the table conveniently, is also presented. The nucleated fragment was the animal fragment in Nos. 5, 221, 223, 230, 231, 237, 252, 253, 278, 283, 284. The nucleated fragment was the vegetal fragment in Nos. 61, 62, 66, 68, 227, 229, 254, 269, 275, 276, 277, 279, 285.

SIZE OF FRAGMENT AND EXTENT OF DEVELOPMENT [1]

As an introduction to the consideration of the size of fragments and the extent of their development, it is essential to discuss the probable volume of small fragments from a theoretical standpoint.

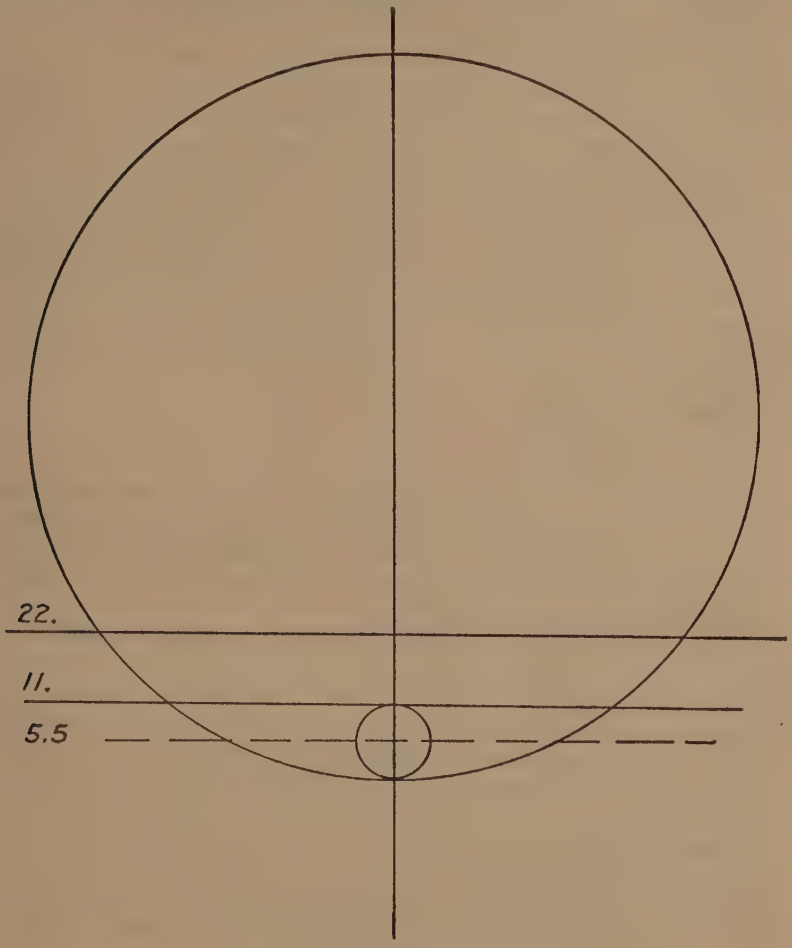


Fig. 50

The most accurate method of determining the size of a small fragment is by computing its volume as a segment of a sphere. Attempts to determine the volume of a fragment after it has been cut from the egg are unsatisfac-

[1] The authors are indebted to Professor Clara Latimer Bacon for advice concerning the formulæ used in this section of the paper.

tory if the fragment fails to round up quickly to a spherical or an ellipsoidal form. Even under the most favorable conditions it is difficult to make the necessary measurements of three axes.

It is easy, on the other hand, to draw a circle representing the egg and to note on this the position of the plane of section when the egg has been oriented and it has been decided where to make the cut. A natural unit of measurement is the diameter of the nucleus. The relative volume of the fragments may be computed from this sketch.

A brief consideration is convincing in its demonstration that with a knowledge of the diameter of the egg and of the nucleus, the determination of the volume of a small fragment may be made with accuracy.

For purposes of illustration we will regard the egg and nucleus as spheres whose diameters measure respectively 110 and 11 microns, and will compute the volume of four spherical segments, one whose height is half that of the diameter of the nucleus, a second whose height is equal to the diameter of the nucleus, a third whose height is one and one-half times that of the diameter of the nucleus, and a fourth whose height is twice that of the diameter of the nucleus.

Diameter of large sphere 110

Diameter of small sphere 11

(1) Volume of large sphere $4/3\pi r^3=696911.6$

Volume of segment of large sphere, height 5.5, *i.e.*, half the diameter of the small sphere, computed by the formula $1/3\pi(3r-h)h^2=5052.61$. Volume of large fragment by subtraction equals 691858.99. Relative volume of large fragment to small fragment approximately 137 : 1. Relative volume of egg to small fragment 138 : 1.

(2) Volume of segment of large sphere, height 11, *i.e.*, the diameter of the small sphere, equals 19513.52. Relative volume of large sphere to segment equals 35.7 : 1.

(3) Volume of segment of large sphere, height 16.5 *i.e.*, one and one-half times the diameter of the small sphere equals 39486.38. Relative volume of large sphere to segment equals 16.6 : 1.

(4) Volume of segment of large sphere, height 22, *i.e.*, twice the diameter of the small sphere, equals 72478.8. Relative volume of large sphere to segment equals 9.6 : 1.

Attention is especially caught by the great difference in volume of segments with a height of 5.5 and 11, producing in the first instance a ratio of 138 : 1, and in the second 35.7 : 1, between volume of sphere and of segment.

The eggs of *Lytechinus* are usually ellipsoidal in form, with axes varying within the limits of $110\times100\times100$ and $120\times110\times110$; the axes of the nucleus vary from $11\times10\times10$ to $12\times11\times11$. It is therefore of interest to compute the volume of segments of an ellipsoid of these dimensions, and those of two heights, 11 and 22, are here included.

Computed as an ellipsoid
Egg axes 110×100×100
Nuclear axes 11×10×10
Segment of ellipsoid, height 11.
Solving by the formula:

$$V = 100/121\pi \int_{44}^{55} (3025 - x^2)\, dx$$

$V = 16126.01$
Volume of egg equals 575960.
Relative volume of two fragments equals 34.7 : 1.

The small fragment has approximately 1/36 the volume of the egg. The true values are 35.716 : 1.

Segment of ellipsoid, height 22.
Solving by the formula:

$$V = 100/121\pi \int_{33}^{55} (3025 - x^2)\, dx$$

$V = 59899.23$

Relative volume of two fragments 8.6 to 1. The small fragment has approximately 1/10 the volume of the egg. The true values are 9.615 : 1.

With the values selected the relative volumes of the spherical and ellipsoidal segments are the same to the first two decimal places.

It is also of interest to calculate the relative volume of theoretical fragments. A nucleated spherical fragment having a diameter twice that of the nucleus, *i.e.* of 22 microns, seems conceivable. Such a fragment might have the nucleus in the center and a layer of cytoplasm 5.5 microns thick surrounding it. Its volume would be about 1/124 that of the whole egg. A nucleated fragment of this volume could not be obtained by a straight cut without compression of the egg or displacement of the nucleus.

A spherical fragment having a diameter three times that of the nucleus, *i.e.* 33 microns, would have about 1/36 the volume of the whole egg. This is slightly smaller than could be made without compression of the egg, or slight displacement of the nucleus, if the cut were straight.

Further, even when the nucleus is close to the surface of the egg it is not actually in contact with the egg membrane, so that the height of the usual nucleated segment will be greater than the diameter of the nucleus. The smallest probable nucleated egg fragment, cut without compression of the egg or displacement of the nucleus would be from a segment with a height of 12 microns. This fragment would have about 1/30 the volume of the entire egg.

It is with these facts in mind that from all of the data at our command, including measurements made with a micrometer eye piece, sketches made from both fragments of a pair after they had rounded up, and sketches of the whole egg in which the position of the plane of section is indicated, we have calculated the volume of our fragments.

Non-nucleated fragments from segments whose heights were less than that of the nuclear diameter, in some instances not more than half its diameter, which were little more than shreds of the cortical layer, were obtained at various times. These remained irregular in form and did not become fertilized.

The smallest fragment from which we obtained a pluteus was slightly less than 1/4 of the egg in volume (No. 54), 1/4.4, to be exact. The smallest fragment that gave us a gastrula had 1/11 the volume of the egg (No. 253). The smallest fragment that produced a blastula had 1/21 of the volume of the egg (No. 275). It will be seen from inspection of the table presenting the history of reared fragments that fragments having 1/11, 1/17 and 1/21 the volume of the egg, reached the blastula stage and did not develop beyond it.

The table should be read with the normal rate of development in mind. From eggs fertilized and allowed to develop in sea-water of a temperature of 28° C., blastulæ begin to swim in 5 hours and 20 minutes; proliferation of mesenchyme begins in 7 hours and 45 minutes; gastrulation begins in 8 hours and 45 minutes; the formation of skeletal spicules begins in 14 hours; and the stage of a young pluteus with post-oral arms is reached in 24 hours.

Driesch ('00, page 390) holds that development is retarded as germinal value decreases. For the development of *Echinus* he gives the following table:

Echinus: Age in hours	Germinal value			
	1/1	1/2	1/4	1/8
24.5	Gastrula...	Early gastrula.....	Very early gastrula..	Blastula.
49	Pluteus....	Early pluteus short arm rods.	Gastrula, with skeletal spicules.	Blastula and gastrula.
72.5	Pluteus....	Pluteus.....	Early pluteus short arm rods.	Gastrula with skeletal spicules.
97.5	Pluteus....	Pluteus.....	Pluteus.....	Gastrula with skeletal spicules.

Our own results do not agree wholly with this. In our No. 54, for example, which would be placed in the 1/4 class, the two fragments developed at the same rate throughout. They were inseminated on July 23 at 11^h 45^m; both were swimming at 6^h 5^m, the smaller fragment

(nucleated) having 4 free mesenchyme cells, the larger fragment having 12 free mesenchyme cells in the blastocœle. On July 24 at 9^h 45^m, both were young plutei; on July 25 they were similar older plutei, their differences being wholly in size. A 1/10 fragment was in the stage of gastrula with skeletal spicules in 18 hours. It should be noted, however, that the development of *Lytechinus* is normally more rapid than that of *Echinus*.

It is probably safe to conclude that blastulæ more than 20 hours old (Nos. 299, 23, 25, 60, 22, etc.) have reached the limit of their possible development. It would probably be safe to say that if gastrulation has not begun in a larva from a fragment of a *Lytechinus* egg within 14 hours after fertilization it will not occur, no matter how long the larva may be kept. On the other hand there would seem to be no reason for supposing that a larva which is in the gastrula stage at 22 hours, might not go on to a more advanced stage of development, although in our material we find one (No. 231) that was still in the stage of a gastrula with skeletal spicules at 46 hours.

The region from which the fragment comes seems to be of importance when extent of development is concerned. Our records show that while we succeeded in rearing gastrulæ from vegetal fragments of 1/11 the volume of the egg, none of the larvæ from fragments of equal size from the region of the animal pole developed beyond the stage of blastulæ with mesenchyme.

The smallest fragment that developed to a swimming larva (blastula with four mesenchyme cells), No. 275, had a volume of about 1/21 that of the egg. It is of interest to consider the stage of development reached by other fragments of this and lesser size, which were not reared and therefore do not appear in the table. These are,

No. 232. 1/50-1/40. No fertilization membrane; no development.
No. 272. 1/50-1/40. Irregular cleavage.
No. 233. 1/35. No fertilization membrane; no development.
No. 247. 1/35. Formed fertilization membrane, but did not segment.
No. 197. 1/30. Formed fertilization membrane; abnormal cleavage; cells pressed closely together; cleavage could not be followed.
No. 116. 1/24. Formed fertilization membrane; cleavage to 16 equal cells; no micromeres; was not followed further.
No. 265. 1/18. Formed fertilization membrane, cleaved regularly and formed 4 micromeres.
No. 269. 1/18. Formed fertilization membrane; did not round up perfectly; formed 4 micromeres; form of the 16-cell stage not normal because of the failure of the fragment to become spherical.

Driesch ('00) concluded that in *Echinus* the smallest isolated blastomeres that will gastrulate are those of the 32-cell stage; presumably any of the vegetative blastomeres of this stage except the micromeres; and that the smallest fertilized fragment of an unfertilized egg that will gastrulate is 1/32 of the egg.

His estimates of the germinal value of a larva (blastula) were based on his conclusion that the surface area of the larva is proportional to the germinal value of the egg fragment from which the blastula came. Thus letting O represent the surface of a blastula from a whole egg and o the surface of a larva from one-fourth an egg, the surface of the larva from the one-fourth egg will equal one-fourth the surface of the larva from the whole egg, $o_4 = \frac{O}{4}$, or $o_n = \frac{O}{n}$; while the volumes of a larva from a whole egg (V) and one-fourth egg (v) would be $v_4 = \frac{V}{8}$, or in a general formula $v_n = \frac{V}{\sqrt{n^3}}$.

He concluded that the gastrulæ that Morgan ('95) estimated as having come from fragments of 1/50 to 1/40 the volume of the egg, actually came from fragments having 1/16 the volume of the egg.

The fact should be kept in mind that the values presented in this paper are based on the measurement of individual fragments that were kept under observation until they reached their larval form, while those which have been given by the investigators whose work has been cited were based on calculations on larvæ from mass cultures of fragmented eggs, or on separated blastomeres from mass cultures, the germinal value of larvæ being determined from measurements of larvæ in these cultures.

However, the number of observations that we present is not great enough to serve as a basis for generalization, and we simply present the facts as they stand.

Size of fragment	Extent of development
1/75—1/50	Did not become fertilized.
1/50—1/35	Fertilization, but no regular cleavage.
1/31 (No. 252)—1/24 (No. 116)	Regular cleavage.
1/21 (No. 275)—1/17 (No. 229)	Blastulæ with mesenchyme.
1/11—1/10	Grastulæ.
1/4.4 = (5/22)	Normal pluteus.

Careful consideration of the data shows that stages more advanced than blastulæ with mesenchyme were never obtained from the smallest animal fragments, while gastrulæ were obtained from equally small vegetal fragments and from small fragments made by vertical section, regardless of the presence or absence of the nucleus in the fragment.

DISCUSSION

In normal development the primary mesenchyme arises from the micromeres that are formed as the egg passes from the 8- to the 12-cell stage. The subsequent cleavage of these micromeres suggests a process of differentiation. The first 4 micromeres divide unequally, producing 4 "small" micromeres which lie centrally placed. Each of the remaining portions then divides equally, a ring of 8 cells surrounding the 4 "small" micromeres thus being formed. This process of cleavage may be concerned with the separation of primary from secondary mesenchyme, but there is no evidence on which to base such a conclusion.

The experimental evidence given in this paper makes it clear that the formation of micromeres is not necessarily antecedent to the formation of mesenchyme. This statement holds both for egg-fragments and for whole eggs in which the formation of micromeres has been inhibited.

The evidence also shows that a spherical segment may be cut from any region of the egg without necessarily inhibiting the formation of micromeres on the fragment remaining. Therefore the idea of complete localization of either micromere-forming or mesenchyme-forming material can not be held.

If the animal half of an egg always failed to form mesenchyme after the removal of the vegetal half, the case for prelocalization of mesenchyme-forming material in the vegetal half of the unfertilized egg would be reasonably complete. The fact that an animal half *may* form mesenchyme does not, however, prove that there was no prelocalization. It does show that there could not have been complete localization.

The same thing is shown by experiments of Driesch ('93) and Zoja ('95). Driesch for *Echinus* and Zoja for *Strongylocentrotus* found that when the micromeres were removed from the 16-cell stage of the cleaving egg the remaining macromeres and mesomeres might produce a perfect pluteus. Zoja's observation is of especial significance for the reason that the micromeres were separated with a needle and observed, all four having been isolated in such a way that there was no doubt of the observation (l. c. page 26). No suggestion of "de-differentiation" as a result of fragmentation by shaking can enter here.

It is thus evident that even after the formation of the micromeres in regular cleavage the other blastomeres retain the ability to produce mesenchyme. In other words, the mesenchyme-producing substance is not completely localized in the micromeres.

Further, we have shown that in most of our fragments the first two planes of cleavage were at right angles to the plane of section and that the micromeres were formed on the surface of section at the intersection of these two planes. It is therefore evident, in the fragments derived by vertical section, that these lie at one end of an axis which forms a right angle with

the primary axis of the egg. A new axis has been established. Differentiation can not be questioned here. If mesenchyme-forming substance is distributed uniformly throughout the egg it can not be said to be prelocalized. If it is formed from an undifferentiated mass it must be said to be differentiated.

The results described in this paper have compelled the conclusion that in the unfertilized egg of *Lytechinus* there is an animal polar cap and a superficial layer of ectoderm-forming material surrounding a core of undifferentiated material that is potential endoderm, primary and secondary mesenchyme, and mesoderm. All of the evidence points to development by *differentiation* rather than by *distribution* of prelocalized substances.

SUMMARY

The following conclusions have been drawn from the investigation on *Lytechinus* eggs cut with precision into two fragments which were then fertilized and allowed to develop.

(1) Animal and vegetal halves, the halves obtained by vertical section, and the halves obtained by random sectioning, all may give rise to blastulæ with mesenchyme, which may develop in turn to plutei.

(2) There is not a complete segregation of primary mesenchyme in the mature, unfertilized egg.

(3) With a few exceptions the first two planes of cleavage were at right angles to the surface of section, regardless of the orientation of the egg at the time of cutting and a new polar axis was established.

(4) The number and distribution of micromeres is not dependent on the n or the $2n$ quantity of nuclear material.

(5) The formation of primary mesenchyme is not dependent on the formation of micromeres.

(6) Fragments that give rise to no micromeres, or to one, or to two, or to three, or to four, during their cleavage may all give rise to larvæ with mesenchyme.

(7) Fragments varying in size down to 1/20 the volume of the entire egg, regardless of the region of the surface involved, may give rise to a normal blastula with mesenchyme.

(8) The experiments have indicated that prior to fertilization a mesenchyme-endoderm forming substance has a uniform distribution through about 19/20 of the egg.

(9) The animal polar cap seems to contain only ectoderm-forming material.

(10) Inconclusive evidence that there is incomplete prelocalization of endoderm-mesenchyme-forming material in the egg is given by the cleavage pattern of large fragments that have been obtained by cutting the egg in a plane below that of the equator. In fragments of this kind the cells that produce the micromeres are smaller than those that become mesomeres.

(11) More conclusive evidence that there is prelocalized endoderm-forming material is afforded by the fact that small animal fragments develop only to blastulæ, while vegetal fragments of the same size develop to gastrulæ.

(12) The tendency of the first cleavage amphiaster to lie temporarily in the short axis of fragments obtained by vertical section suggests the persistence of an established, although modifiable, organization.

(13) Asymmetry of the cleavage pattern of some fragments obtained by diagonal section, contrasted with symmetrical cleavage of fragments obtained by horizontal section, suggests, but does not demonstrate, a bilaterally symmetrical organization of the egg.

(14) Whole eggs were found to give the same varieties of atypical cleavage as those observed in fragments.

(15) The ability of a small fragment to form a gastrula is not dependent on the presence or absence of the egg nucleus in the fragment, or on the closeness of the nucleus to the plane of section.

(16) Fragments of 1/75 to 1/50 of the volume of the egg did not become fertilized when inseminated. Those of 1/50 to 1/35 the volume of the egg became fertilized but did not segment regularly; those of 1/31 to 1/24 gave regular cleavage; those of 1/21 to 1/17 formed blastulæ with mesenchyme; those of 1/11 to 1/10 formed gastrulæ.

(17) The smallest fragment that formed a pluteus was of slightly less than 1/4 the volume of the egg.

(18) The distinction between differentiation of organ-forming substances and their prelocalization is emphasized.

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APPENDIX

DEVELOPMENT OF LYTECHINUS EGG FRAGMENTS, 1924

At the conclusion of the work in 1926, when the tabulation and analysis of results were begun, each pair of fragments that formed membranes after insemination, or one or both of which began cleavage despite the fact that a membrane was not well-separated, was given a serial number, this number replacing the daily experiment number. These serial numbers ran from 1 to 80 for the work of 1924, and from 1 to 285 for that of 1926, and replaced numbers aggregating 122 and 451. The detailed account is presented as a record of value for its information on individual cases.

N and NN are used as abbreviations for nucleated and non-nucleated fragments. The word non-nucleated is used instead of enucleated for the reason that "enucleated" should apply to fragments from which the nucleus has been removed.

When the expressions 2-cell, 4-cell, etc., are preceded by the word "into," the process of division was seen as it was occurring at the time recorded. If the statement is simply 2-cell, or in 2-cell, followed by the time of the observation, nothing more than the stage seen at the time stated is implied. The words "upper" and "lower" have been used instead of the longer expressions "four cells of the animal half" and "four cells of the vegetal half" of the egg in the 8-cell stage. The words "macromeres" and "mesomeres" do not apply until after the "micromeres" have been formed.

The ratios 1 : 1, 1 : 2, etc., given after the statement of type of section, are statements of the relative volume of nucleated to non-nucleated fragment. The order is always N : NN.

LYTECHINUS FRAGMENTS INSEMINATED WITH LYTECHINUS SPERM SERIES OF 1924

June 25 No. 1. Random section. Relative volume N : NN = 1 : 1.
Inseminated 3^h 30^m. Membranes formed.
N into 8 cells 4^h 40^m.
NN into 8 cells 4^h 40^m.
Control (whole egg) not dividing; at 5^h 00^m divided into about 16 cells.
All polyspermy.

June 26 No. 2. Random section. 1 : 1.
Inseminated 8^h 45^m. Membranes formed.
N into regular 8-cell 10^h 10^m.
NN did not divide.

June 27 No. 3. Random section. 1 : 1. (Figure 3.)
Inseminated 11^h 56^m. Membranes formed.
N beginning constriction 12^h 37^m; complete 2-cell 12^h 38^m.
NN beginning constriction 12^h 50^m; complete 2-cell 12^h 55^m.

No. 4. Random section. 1 : 2.
Inseminated 3^h 45^m. Membranes formed.
N divided into 3-cells 4^h 52^m.
NN regular 2-cell 5^h 01^m; 3 cells 5^h 40^m; one of the cells of the 2-cell stage did not divide.

July 5 No. 5. Horizontal section. 1 : 1.
Inseminated 11^h 42^m. Membranes formed.
N animal half; 2-cell 12^h 30^m; irregular at 1^h 15^m and 1^h 45^m; a ball of regular cells 2^h 37^m; slightly irregular 3^h 30^m; regular 6^h 25^m; July 6, 9^h 00^m a.m. active blastula.
NN vegetal half; not divided 12^h 40^m; in regular 2-cell 1^h 10^m; irregular 4-cell 1^h 47^m; many cells, irregular, 3^h 35^m; ball of small cells 6^h 25^m. July 6, 9^h 00^m a.m. abnormal larva, discarded.

No. 6. Horizontal section. 1 : 1.
Inseminated 1^h 10^m. Membranes formed.
N animal half; dividing irregularly 1^h 55^m; irregular mass of cells 3^h 00^m; blastula 6^h 30^m; July 6, 9^h 00^m a.m. dead blastula.
NN vegetal half; did not divide.

No. 7. Random section. 1 : 1.
Inseminated 2^h 28^m. Membranes formed.
N no cleavage.
NN no cleavage.

No. 8. Horizontal section. 1 : 1.
Inseminated 2^h 28^m. Membranes formed.
N animal half; well rounded 3^h 05^m; 4-cell 3^h 45^m; dividing well 6^h 15^m.
NN vegetal half; well rounded 3^h 05^m; 3-cell 3^h 45^m; 4-cell 3^h 46^m; dividing well 6^h 15^m.
July 6, 9^h 00^m a.m. both gastrulæ; July 8, 11^h 00^m a.m.
NN lost; N pluteus without arms. Kept until July 14 but no arms developed.

No. 9. Random section. 1 : 1.
Inseminated 2^h 51^m. Membrane formed.
N many irregular cells 6^h 15^m; July 6, 9^h 00^m a.m. disintegrated.
NN 5 cells 6^h 15^m; July 6, 9^h 00^m a.m. disintegrated.

No. 10. Random section. 1 : 2.
Inseminated 3^h 22^m. Membranes formed.
N divided; July 6, 9^h 00^m disintegrated.
NN July 6, 9^h 00^m a.m. disintegrated.

July 6 No. 11. Random section. 1 : 1.
Inseminated 11^h 19^m. Membranes formed.
N no cleavage.
NN divided into 2 cells only slightly in contact 12^h 40^m; these cells separated at 1^h 35^m, both cells dividing; irregular mass of cells 4^h 20^m; disintegrated 6^h 37^m.

No. 12. Random section. 1 : 1.
Inseminated 11^h 47^m. Membranes formed.
N dividing 1^h 18^m; hollow sphere of cells with 2 cells attached 4^h 10^m; slightly elongated hollow mass 3 cells attached 6^h 40^m; July 7, 8^h 30^m a.m. swimming blastula.
NN unchanged 1^h 18^m; ragged clump of cells 4^h 15^m; spherical mass 6^h 40^m; July 7, 8^h 50^m a.m. inactive mass.

No. 13. Random section. 1 : 1.
Inseminated 12^h 02^m. Membranes formed.
N unchanged 1^h 15^m; irregular string of cells 4^h 07^m; no further development.
NN in 2 cells slightly separated 1^h 15^m; these cells separated and divided independently 1^h 45^m; irregular mass of each 6^h 50^m; July 7, 9^h 00^m a.m. found nothing.

July 6 No. 14. Random section. 1 : 1. From another female.
Inseminated 2^h 09^m. Membranes formed.
N 8 cells 3^h 40^m; irregular group 7^h 05^m; July 7, 9^h 05^m a.m. disintegrated.
NN no change 3^h 40^m; about 6 irregular cells 3^h 52^m; irregular ball 6^h 52^m; July 7, 9^h 06^m a.m. disintegrated.

No. 15. Random section. 1 : 1.
Inseminated 2^h 25^m. Membranes formed.
N 5 cells 3^h 38^m; 5 cells 3^h 53^m; irregular sphere 6^h 57^m; July 7, 9^h 10^m a.m. disintegrated.
NN no change 3^h 38^m; 4 cells 3^h 55^m; opaque mass 6^h 55^m; July 7, 9^h 10^m a.m. disintegrated.

No. 16. Random section. 1 : 2.
Inseminated 2^h 37^m. Membranes, close, but separated.
N no change 3^h 42^m; blastula 7^h 00^m; July 7, 9^h 20^m a.m. disintegrated.
NN 5 cells 3^h 41^m; 3 cells 3^h 50^m; ball of irregular cells 6^h 59^m; July 7, 9^h 20^m a.m. disintegrated.

No. 17. Vertical section. 1 : 1.
Inseminated 3^h 12^m. Membranes formed.
N blastula 7^h 03^m; July 7, 9^h 25^m a.m. disintegrated.
NN July 7, 9^h 25^m disintegrated.

No. 18. Random section. 1 : 1.
Inseminated 11^h 05^m. Membranes formed.
N disintegrating 4^h 20^m.
NN disintegrating 4^h 20^m.

July 7 No. 19. Horizontal section 1 : 2.
Inseminated 11^h 12^m. Membranes formed.
N animal; did not divide.
NN vegetal; formed fine amphiaster and looked about to divide 12^h 07^m; cleavage plane ragged; failed to divide; rounded up again 12^h 18^m; 2 amphiasters 12^h 22^m these being situated at right angles to long axis of original one; then divided slightly irregularly to 4 cells 12^h 40^m; cleavage continued, seemingly bilateral to the plane of the inhibited first cleavage.

No. 20. Random section. 1 : 1. From another lot of eggs.
Inseminated 2^h 15^m. Membranes formed.
N divided regularly 2^h 58^m; 4-cell 3^h 20^m; continued division, but not normally. July 10, pluteus without arms; July 12, no further development.
NN amphiaster 3^h 22^m; excellent 2-cell 3^h 35^m; 4-cell 4^h 00^m; 8-cell 4^h 30^m; upper four cells divided equally giving 12-cell stage 5^h 00^m; lower 4 divided equally 5^h 33^m; micromeres formed 5^h 45^m (delayed formation of micromeres). July 10, 9^h 00^m a.m. could not find.

July 8 No. 21. Random section. 1 : 1.
Inseminated 2^h 22^m. Membranes formed.
N first division complete 3^h 15^m; one cell slightly larger than the other; into 4 cells 3^h 43^m; July 9, 9^h 00^m a.m. good gastrula. July 10, 9^h 00^m a.m. same; July 12, no further development.
NN not divided 3^h 17^m; amphiaster at first in short axis, then in long axis; divided to 2 cells, one slightly larger than the other, 3^h 30^m; blastula 6^h 15^m. July 9, a swimming sphere filled with cells; slight evagination of archenteron. July 10, could find nothing.

July 9 No. 22. Vertical section. 1 : 1.
Inseminated 10^h 36^m.
N fair membrane; not divided at 12^h 08^m; no development.
NN no membrane; hour-glass shaped; membrane covering one half 12^h 00^m; 3^h 40^m irregular lump of rounded fragments; July 10, 8^h 45^m a.m. disintegrated.

No. 23. Vertical section. 1 : 1.
Inseminated 10^h 55^m.
N fair membrane; good blastula 4^h 05^m; July 10, 8^h 50^m a.m. abnormal larva, rotating. July 12, discarded.
NN no membrane; fragmented 4^h 00^m.

No. 24. Vertical section. 2 : 1.
Inseminated 11^h 30^m. Membranes formed.
Two pieces adhering.
N developing 4^h 15^m; July 10, 8^h 45^m a.m. disintegrated.
NN separated from larger piece but did not develop; July 10, 8^h 45^m disintegrated.

No. 25. Vertical section. 1 : 1.
Inseminated 11^h 42^m. No membrane formed.
N sphere of regular cells 4^h 15^m; July 10, 8^h 45^m a.m. swimming blastula.
NN rather irregular sphere of cells 4^h 20^m; irregular mass of cells July 10, 8^h 45^m a.m.

No. 26. Vertical section. 1.5 : 1.
Inseminated 11^h 42^m. Membranes formed.
N perfect 2-cell at 12^h 40^m; to 4-cell 12^h 48^m; swimming 6^h 00^m.
The first division of this egg, as could be seen by the position of the attached piece, was at right angles to what the division would have been in the normal entire egg, *i. e.* it was in the equatorial plane. It resulted in the formation of two blastomeres of slightly unequal size. After the 8-cell stage the 4 cells on the side away from the NN fragment divided again (fig. 7). The 4 cells on the side next NN fragment then gave rise to the micromeres, these being formed at one end of the first furrow.
NN did not divide.

No. 27. Vertical section 1 : 1.
Inseminated 1^h 58^m. Membranes formed.
N 4-cell 3^h 10^m; July 14, pluteus without arms, had skeleton, perfect digestive tract, and enterocœles.
NN did not divide.

No. 28. Vertical section 1 : 1.
Inseminated 2^h 28^m. Membranes formed.
N good sphere, regular cells 4^h 35^m; no further development.
NN no development 4^h 36^m.

No. 29. Vertical section. 1 : 1.
Inseminated 2^h 44^m. No membranes formed.
N rounded up well and showed asters at once. No further development.
NN rounded up well and showed asters at once; 16 cells 4^h 30^m.

No. 30. Vertical section. 1 : 1.
Inseminated 3^h 09^m. Membranes formed.
N and NN adhering to each other.
N divided into 4 cells 3^h 55^m; no further development.
NN dividing 4^h 00^m; no further development. Polyspermy in both.

July 9 No. 31. Vertical section. 1 : 1.
Inseminated $2^h\ 58^m$. Membranes formed.
N in 4-cell stage, one of which is irregular $4^h\ 25^m$; no further development.
NN in 3 well-rounded cells $4^h\ 27^m$; no further development.

July 11 No. 32. Vertical section. 1 : 1.
Inseminated $11^h\ 20^m$. Membranes formed.
N polyspermy; divided to 5 cells $12^h\ 10^m$.
NN polyspermy; divided into 6 cells $12^h\ 12^m$.

No. 33. Vertical section. 1 : 1.
Inseminated $1^h\ 53^m$. Membranes formed.
N polyspermy.
NN polyspermy.

No. 34. Vertical section. 1 : 1.
Inseminated $2^h\ 12^m$. Membranes formed.
N not divided $3^h\ 35^m$.
NN 2-cell $3^h\ 20^m$; 4-cell $3^h\ 55^m$. Protrusion (Figs. 51 a and b).

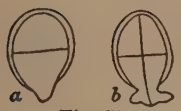

Fig. 51

July 12. No. 35. Vertical section. 1 : 1.
Inseminated $11^h\ 21^m$. Membranes formed.
N polyspermy; divided $12^h\ 20^m$, 5 cells; many cells $1^h\ 20^m$.
NN endoplasmic bud through membrane; 6 cells $1^h\ 15^m$, bud did not divide.

No. 36. Vertical section.
Inseminated $12^h\ 00^m$.
N no record.
NN fine membrane; 2 cells at $1^h\ 10^m$; dividing irregularly at $2^h\ 00^m$. July 14, erratic swimming larva.

No. 37. Vertical section. 1 : 1.
Inseminated $2^h\ 15^m$. Membranes formed.
N dividing to 5 cells $3^h\ 05^m$; July 14, disintegrated.
NN dividing to 4 cells $3^h\ 15^m$; July 14, erratic gastrula.

No. 38. Vertical section. 1 : 1.
Inseminated $2^h\ 30^m$. Membranes formed.
N perfect 2-cell $3^h\ 25^m$; developed to blastula; lost through evaporation of water.
NN amphiaster formed in short axis of fragment. During mitosis the fragment gradually broadened until its axes became nearly equal; at $3^h\ 45^m$ dividing into 2 cells of nearly equal size, division being in the direction of the original long axis of the fragment; July 14 dead gastrula.

No. 39. Vertical section. 1 : 1.
Inseminated $2^h\ 56^m$. Membranes formed.
N did not develop.
NN fragment stuck to dish; endoplasmic bud; disintegrated.

No. 40. Vertical section. 1 : 1.
Inseminated $3^h\ 45^m$. Membranes formed.
N beginning of first division at $4^h\ 27^m$; division complete $4^h\ 30^m$; perfect 2-cell; died July 13 as young pluteus with skeletal rods.
NN first division complete $4^h\ 30^m$; perfect 2-cell; July 14, perfect pluteus, post oral arms well developed.

July 13 No. 41. Horizontal section. 1 : 1.
Inseminated 10^h 56^m. Membranes formed.
N beginning constriction of first division 11^h 51^m. First division completed 11^h 54^m. July 14, gastrula, prism form, good skeleton. July 15, gastrula (fixed on saturated sublimate).
NN 2-cell 12^h 25^m. Did not develop beyond late segmentation.

No. 42. Horizontal section. 1 : 1.
Inseminated 11^h 09^m. Membranes formed.
N beginning constriction of first division 11^h 58^m. First division completed 12^h 00^m. July 14, pluteus with arms and skeleton. July 15, pluteus (fixed in saturated sublimate).
NN in 2-cell 12^h 35^m; July 14, dead gastrula with small triradiate spicules.

No. 43. Horizontal section. 1 : 1.
Inseminated 11^h 43^m. Membranes formed.
N beginning constriction of first division 12^h 20^m. First division completed 12^h 23^m; July 14, disintegrated.
NN divided to 2 blastomeres of slightly unequal size. July 14, disintegrated.

No. 44. Horizontal section. 1 : 1.
Inseminated 12^h 01^m.
N divided to 3 cells about 1^h 20^m; July 14, late gastrula, prism form; July 15, gastrula (fixed in saturated sublimate).

No. 45. Horizontal section. 1 : 1.
Inseminated 2^h 56^m. Poor membranes.
NN segmented; July 14, blastula with mesenchyme.
NN segmented; July 14, blastula with mesenchyme.

No. 46. Horizontal section. 1 : 1.
Inseminated 3^h 21^m. Membranes formed.
N segmented; July 14, disintegrated.
NN found in 2-cell 4^h 10^m; dividing to 4 at 4^h 42^m; July 14, died during late segmentation.

No. 47. Horizontal section. 1 : 1.
Inseminated 4^h 12^m. Membranes formed.
N July 14, fine pluteus with skeleton; July 15, fine pluteus (fixed in saturated sublimate).
NN July 14, whirling ball, slightly prismatic in form; pigmented.

July 14 No. 48. Random section. 1 : 1.
Inseminated 2^h 59^m. Membranes formed.
Both fragments were studied on slide under 4 D. from 3^h 04^m to 3^h 50^m for stages of mitosis (Fig. 52).

July 15 No. 49. Horizontal section. 1 : 1.
Inseminated 2^h 39^m. Membranes formed. Both fragments were studied on slide under 4 D for stages of mitosis.

No. 50. Horizontal section. 1 : 1.
Inseminated 4^h 13^m. Membranes formed.
Both fragments were studied on slide under 4 D for stages of mitosis.

July 16 No. 51. Horizontal section. 1 : 1.
Inseminated 11^h 09^m. Membranes formed.
N studied under 4 D for stages of mitosis.
NN not studied.

July 16 No. 52. Random section. 1 : 1.
Inseminated 3^h 10^m. Membranes formed.
N not studied.
NN studied under 4 D for stages of mitosis.

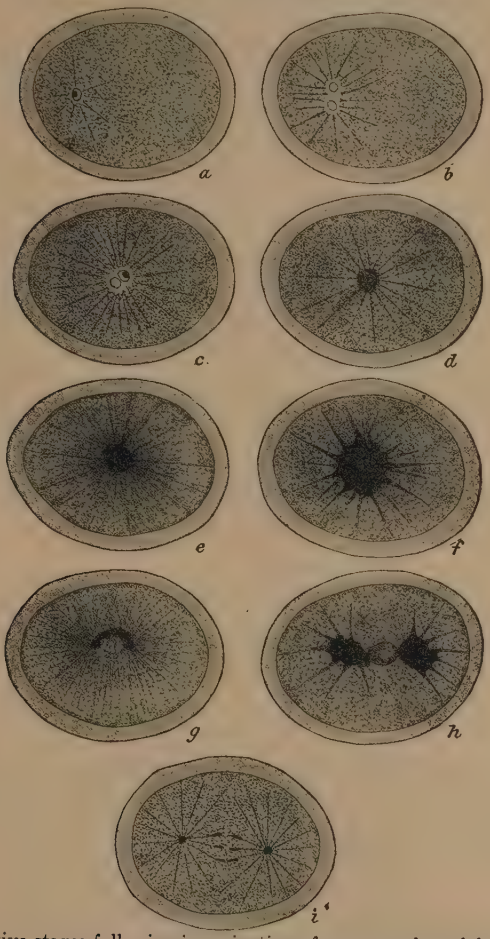

Fig. 52—Consecutive stages following insemination of a non-nucleated fragment. In the reproduction of the sketches the clear perivitelline space appears as a grey border.

July 23 No. 53. Vertical section. 1 : 1.

Inseminated 11^h 25^m. Membranes were not separated, but sperm asters were seen in both fragments.

N cleavage regular; 32 cells 2^h 00^m; swimming blastula with mesenchyme 6^h 00^m; July 24, 9^h 00^m a.m. dead gastrula with 2 triradiate spicules.

NN normal 4-cell 1^h 00^m. Cleaving by equal division of all cells, (no micromeres) 2^h 05^m; swimming blastula with mesenchyme 6^h 30^m; July 24, 9^h 00^m a.m. active gastrula with skeletal spicules. July 25, disintegrated.

July 23 No. 54. Horizontal section 1 : 3.4.
Inseminated $11^h\ 45^m$. Membranes formed. (fig. 38 a and b)
N cleaving regularly at $2^h\ 22^m$; same at $3^h\ 55^m$. Could not be certain of number of micromeres. Blastula with 4 free mesenchyme cells at $6^h\ 05^m$; July 24, $9^h\ 45^m$ a.m. pluteus; July 26, normal pluteus.
NN cleaving regularly at $2^h\ 22^m$; same at $3^h\ 55^m$; could not be sure of presence of micromeres; blastula with 12 mesenchyme cells at $6^h\ 05^m$; July 24, $9^h\ 45^m$ a.m. pluteus; July 26, normal pluteus.

No. 55. Vertical section. 1 : 1.
Inseminated $12^h\ 05^m$. Membranes not well separated but sperm asters seen.
N in 2-cell $1^h\ 05^m$. Blastula without mesenchyme $6^h\ 35^m$; July 24, $9^h\ 20^m$ a.m. prism gastrula; July 25, lost.
NN blastula with mesenchyme $6^h\ 35^m$; July 24, $9^h\ 20^m$ a.m. dead.

No. 56. Vertical section. 1 : 1.7.
Inseminated $12^h\ 20^m$. Membranes formed. (fig. 39 a and b)
N in 2-cell $1^h\ 10^m$; into 4-cell $1^h\ 30^m$ continued to divide well. Could not make satisfactory observations on micromeres. July 25, pluteus with poorly developed arms, good body skeleton, normal digestive tract and pigmentation. Same on July 26.
NN into 2-cell at $1^h\ 30^m$. Blastula with mesenchyme $6^h\ 50^m$; July 24, $9^h\ 25^m$ a.m. late prism gastrula. July 25, pluteus with poorly developed arms, good body skeleton, normal digestive tract and pigmentation. Identical with pluteus from N; July 26, same as on July 25; no arms.

No. 57. Horizontal section. 1 : 1.
Inseminated $2^h\ 11^m$.
N did not segment.
NN $7^h\ 00^m$ blastula with thickened posterior wall, but no mesenchyme. July 24, $8^h\ 00^m$ a.m. abnormal gastrula; gastrocœle filled with cells; has 2 triradiate spicules; some of cells in gastrocœle are very large; archenteron short.

No. 58. Vertical section. 1 : 1. Cut between polar bodies.
Inseminated $2^h\ 25^m$. Membranes formed.
N normal 8-cell $3^h\ 50^m$; 4 micromeres formed at $4^h\ 05^m$. Blastula with mesenchyme at $7^h\ 05^m$. July 24, $2^h\ 14^m$ p.m. normal prism gastrula. July 25, $8^h\ 30^m$ a.m. pluteus, arms poorly developed. July 26, $8^h\ 00^m$ a.m. pluteus, arms poorly developed.
NN from 2- to 4-cell $3^h\ 52^m$; normal 8-cell. The cleavage of the fragment was followed closely as it passed from the 8-cell stage. Studied on slide under cover. Division continued to about 28 cells before fragment was injured by pressure. No micromeres were seen.

No. 59. Horizontal section. 1 : 1.
Inseminated $10^h\ 19^m$. Membranes formed.
N first cleavage completed $10^h\ 59^m$. July 25, $9^h\ 00^m$ a.m. normal late prism gastrula. July 26, $9^h\ 00^m$ a.m. pluteus without arms; good body skeleton.
NN did not segment.

July 23 No. 60. Vertical section. 1 : 1.
Inseminated $10^h\ 42^m$. Membranes formed.
N in 2-cell $11^h\ 25^m$; active blastula with mesenchyme $3^h\ 40^m$. July 25, $9^h\ 00^m$ a.m. lost.
NN into 2-cell $11^h\ 35^m$; inactive blastula with mesenchyme $3^h\ 40^m$. July 25, $9^h\ 00^m$ a.m. active gastrula with triradiate spicules. July 26, $8^h\ 00^m$ a.m. young pluteus with normal skeleton.

No. 61. Horizontal section. 1 : 1. N is vegetative half.
Inseminated $11^h\ 07^m$.
N in 2-cell $11^h\ 50^m$; active blastula $6^h\ 00^m$. July 25, $9^h\ 00^m$ a.m. young pluteus, arms not yet grown; digestive tract normal. July 26, good pluteus without arms, slowly active.
NN in 2-cell $11^h\ 35^m$; active bastula $6^h\ 00^m$. July 26, normal pluteus with arms, very active.

No. 62. Horizontal section. 1 : 1. N is vegetative half.
Inseminated $11^h\ 49^m$. N formed membranes.
N disintegrated $12^h\ 50^m$.
NN in 2-cell $12^h\ 50^m$; good sphere but not yet swimming at $6^h\ 00^m$. July 25, $9^h\ 00^m$ a.m. gastrula with skeletal spicules; some abnormal cells in gastrocœle.

No. 63. Vertical section. 1 : 1.
Inseminated $12^h\ 50^m$. No membranes formed.
Cleavage in both irregular. Discarded.

No. 64. Vertical section. 1 : 1.
Inseminated $1^h\ 50^m$. Membranes formed.
N into 2 cells $2^h\ 40^m$. Divided twice regularly; then became abnormal.
NN did not divide.

No. 65. Horizontal section. 1 : 1.
Inseminated $2^h\ 25^m$.
N segmentation normal; could not see micromeres; about to begin swimming at $6^h\ 00^m$. July 25, could not find larva.
NN record identical with that for N.

No. 66. Horizontal section. 1 : 1. N is animal half.
Inseminated $2^h\ 36^m$.
N lost.
NN cleaved regularly; formed 4 micromeres; proliferation of mesenchyme beginning at $6^h\ 30^m$. July 25, $9^h\ 00^m$ a.m. prism gastrula with pigment cells. July 26, $9^h\ 00^m$ a.m. active pluteus, defective in that there is a median post oral arm, containing 2 arm rods. No other post oral arms.

No. 67. Vertical section 1 : 1.
Inseminated $3^h\ 20^m$. Membranes formed.
N and NN joined along plane of section. Single membrane about both. The two pieces, although united, cleaved independently. At $6^h\ 30^m$ looked as though they would produce a single good blastula. July 25, an irregular rotating mass.

No. 68. Horizontal section. 1 : 1. N is vegetative half.
Inseminated $3^h\ 51^m$.
N segmenting regularly at $6^h\ 00^m$. July 25, $9^h\ 00^m$ a.m. evaginate gastrula. July 26, $8^h\ 00^m$ a.m. active abnormal pluteus; skeleton good; pigment cells.
NN disintegrated at $6^h\ 00^m$.

July 25 No. 69. Vertical section. 1 : 15.
Inseminated $12^h\ 40^m$.
N in 2-cell at $1^h\ 35^m$, into 4-cell at $2^h\ 05^m$, the second plane of cleavage being parallel to the cut surface. July 26, $8^h\ 00^m$ a.m lost.
NN in 2-cell $1^h\ 35^m$. July 26, $8^h\ 00^m$ a.m. fairly active, early gastrula.

July 25 No. 70. Horizontal section. 1 : 1.
Inseminated $2^h\ 26^m$. Membrane formed.
N into 2-cell $3^h\ 05^m$, then returned to undivided condition. Again divided to 2 cells $3^h\ 32^m$; and to 4 cells $3^h\ 34^m$.
NN into 2-cells $3^h\ 37^m$.

No. 71. Vertical section. 2 : 1. Fragments in contact.
Inseminated $2^h\ 42^m$. Membranes formed.
N divided $3^h\ 30^m$. Fragments fused, possibly because of jar. No further development.
NN fused with N.

No. 72. Horizontal section. 1 : 1. N is vegetative half.
Inseminated $3^h\ 00^m$.
N and NN fused. No further division.

No. 73. Vertical section. 1 : 1. Cut between polar bodies.
Inseminated $3^h\ 00^m$.
N into 2-cell $3^h\ 50^m$.
NN into 2-cell $4^h\ 05^m$.
July 26, $8^h\ 00^m$ a.m. both lost.

No. 74. Vertical section. 1 : 1.
Inseminated $4^h\ 14^m$.
N and NN segmented. July 26, $8^h\ 00^m$ a.m. found neither.

July 26 No. 75. Random section. 1 : 1.
Inseminated $12^h\ 04^m$.
N and NN into 2 cell $12^h\ 45^m$.

No. 76. Vertical section. 1 : 1.
Inseminated $1^h\ 00^m$.
Fragments fused. No development.

No. 77. Vertical section. 1 : 1.
Inseminated $1^h\ 25^m$. Membranes formed.
N in normal 4 cell $2^h\ 30^m$; 8 cell $2^h\ 50^m$; formed 4 micromeres $3^h\ 05^m$. July 27, gastrula with triradiate spicules.
NN in 2-cell $2^h\ 30^m$; 8-cell $2^h\ 55^m$; possibly formed 4 micromeres at $3^h\ 25^m$. Fragment was injured in manipulation before observation was completed; died during the night.

No. 78. Random section. 1 : 1.
Inseminated $2^h\ 00^m$. No membranes formed. Both had sperm asters. Did not segment normally.

No. 79. Horizontal section. 1 : 1.
Inseminated $2^h\ 40^m$. Membranes formed.
NN only segmented. July 27, $10^h\ 00^m$ a.m. abnormal blastula.

No. 80. Vertical section. 1 : 1.
Inseminated $3^h\ 38^m$.
N in 2-cell $4^h\ 20^m$. July 27, $10^h\ 00^m$ a.m. normal gastrula with skeletal spicules.
NN into 2-cell $4^h\ 25^m$. July 27, $10^h\ 00^m$ a.m. normal gastrula with skeletal spicules.

DEVELOPMENT OF LYTECHINOUS EGG FRAGMENT, 1926

The signs ⊥ and ‖ have been used as abbreviations for the expressions "plane of section perpendicular to—" and "plane of section parallel to the line joining the nucleus with the center of the egg."

Fertilization membranes were formed on both fragments unless otherwise stated.

All of the exceptions to the observation that the first cleavage was at right angles to the surface of section are mentioned. In the condensation of the data on observations for publication the observations on 179 of the 285 pairs recorded are presented. The 106 pairs omitted are non-significant as concerns formation of micromeres or reared larvæ. The pairs omitted formed fertilization membranes and then either formed protrusions, cleaved irregularly, underwent cytolysis during early cleavage, or were followed only to a 2- or 4-cell stage.

June 17 No. 1. Plane of section vertical. 1 : 1. Inseminated $12^h\ 10^m$.
N 4 micromeres.
NN no micromeres.
Both blastulæ with mesenchyme $6^h\ 20^m$.

June 19 No. 15. ⊥ 1 : 1. Inseminated $2^h\ 23^m$.
N regular 8; 4 micromeres forming $4^h\ 25^m$; upper 4 then divided.
NN irregular cleavage.

No. 16. ⊥ and horizontal 1 : 1.
Both regular cleavage.
Both formed 4 micromeres.

June 20 No. 21. ⊥ 10 : 1. Inseminated $10^h\ 44^m$.
N in 2-cell $11^h\ 25^m$; in 4-cell $11^h\ 45^m$; 8-cell $12^h\ 00^m$; 4 micromeres $12^h\ 15^m$.
NN in 2-cell $11^h\ 45^m$; in 4-cell $12^h\ 00^m$; 4 micromeres $1^h\ 25^m$.

No. 25. ⊥ 1 : 1. Inseminated $11^h\ 16^m$.
N dividing $11^h\ 59^m$; formed 4 micromeres.
NN dividing $12^h\ 00^m$; divided unequally; 4-cell consisted of 2 large and 2 small cells.

No. 27. ‖ 1 : 1. Inseminated $2^h\ 35^m$.
N polyspermy; first division $3^h\ 26^m$ gave 4 cells.
NN into 2 cells $3^h\ 37^m$; into 4 cells $4^h\ 03^m$; regular 8-cell; 4 micromeres formed $4^h\ 10^m$.

June 21 No. 31. ‖ 1 : 1. Inseminated $10^h\ 25^m$.
N in 2 cells $11^h\ 29^m$.
NN in 2 irregular cells $11^h\ 50^m$.
Both in 16 cells $1^h\ 50^m$. Equal division of all cells; no micromeres.

No. 32. ‖ N slightly smaller than NN. Inseminated $10^h\ 35^m$.
N fertilized; did not develop.
NN into 2 cells $11^h\ 51^m$; in 16 cells $2^h\ 30^m$.
Equal division of all cells; no micromeres.

No. 34. ‖ 1 : 1. Inseminated $10^h\ 46^m$.
Both with amphiaster and protrusion $11^h\ 40^m$.
N in 2-cell $11^h\ 53^m$; with 3 micromeres $2^h\ 45^m$.
NN 16 cells; no micromeres $2^h\ 45^m$.

No. 35. ‖ 1 : 1. Inseminated $11^h\ 12^m$.
N regular cleavage; no micromeres.
NN failed to divide after 2-cell.

June 22 No. 36. ‖ 2 : 1. Inseminated $10^h\ 26^m$.
N in 2-cell $11^h\ 20^m$; in 4-cell $11^h\ 30^m$; normal 8-cell $11^h\ 52^m$; 16 cells, no micromeres.
NN in 2-cell $11^h\ 20^m$; normal 4-cell $11^h\ 35^m$; normal 8-cell $11^h\ 58^m$; 16-cells, no micromeres.

June 22 No. 37. ‖ 1 : 1. Inseminated 10^h 50^m.
N in 2-cell 11^h 31^m; 4 micromeres 12^h 55^m.
NN amphiaster 11^h 42^m; seem to be 4 micromeres 1 : 10.

No. 39. ‖ and vertical 2 : 1. Inseminated 11^h 10^m.
Both have amphiasters 11^h 45^m.
N 4 micromeres 1^h 45^m.
NN did not develop.

No. 40. ‖ 2 : 1. Inseminated 11^h 26^m.
N in 16-cell stage with 4 micromeres 2^h 05^m.
NN cleaved finely but could not be sure concerning micromeres.

No. 43. ‖ 1 :1. Inseminated 12^h 05^m.
Both formed perfect 8-cell stages.
N formed 2 micromeres.
NN had formed no micromeres at 4^h 00^m.

June 23 No. 44. ‖ 4 : 1. Inseminated 11^h 10^m.
N 4 micromeres at 1^h 00^m.
NN 16 cells; cleavage not in 2 tiers of 8 cells; no micromeres.

June 24 No. 53. ⊥ 1 : 4. Inseminated 3^h 50^m.
N into 2 cells 4^h 55^m; in 8 cells 5^h 40^m. Could not be sure of later cleavage.
NN into 2 cells 4^h 55^m; in 4-cell 5^h 35^m; regular 8-cell. Could not be sure of later cleavage.

No. 54. ⊥ 1 : 15. Inseminated 4^h 00^m.
N 2-cell 5^h 00^m; 8-cell 5^h 45^m; 4 micromeres 5^h 55^m.
NN 2-cell 5^h 00^m; 8-cell 5^h 45^m; 4 micromeres 5^h 55^m.

No. 55. ⊥ 1 : 20. Inseminated 4^h 04^m.
Both in 2 equal cells 5^h 00^m.
Both in 8 regular cells 5^h 47^m.
NN 4 micromeres 6^h 15^m.
N too dark to see 6^h 35^m.

No. 56. ⊥ 1 : 1. Inseminated 4^h 20^m.
N in 2 equal cells 5^h 03^m; in 8-cell 5^h 50^m.
Too dark to see micromeres 6^h 35^m.
NN fine amphiaster 5^h 04^m; in 2 slightly unequal cells 5^h 50^m; later divisions irregular.

June 25 No. 57. ⊥ 1 : 4. Inseminated 10^h 40^m.
N formed 4 micromeres, 4 macromeres, 8 mesomeres.
NN delayed division in one of the first two blastomeres giving a 3-cell 12^h 00^m; 5-cell at 12^h 30^m one of the first blastomeres not having divided although an amphiaster was present. At 12^h 35^m 4 small cells, micromeres (?) formed; 4 macromeres, 8 mesomeres.

No. 61. ⊥ 1 : 10. Inseminated 11^h 36^m.
N 4-cell 12^h 55^m.
NN 2-cell 12^h 55^m; regular 4; regular 8; 2 micromeres formed.

No. 63. ⊥ 1 : 8. Inseminated 11^h 55^m.
N fertilized; did not divide.
NN formed 4 micromeres.

No. 65. ⊥ 1 : 1. Inseminated 3^h 37^m.
N in 2-cell 4^h 20^m; 4-cell 4^h 55^m; 3 micromeres formed.
NN amphiaster 4^h 20^m; 2-cell 4^h 55^m; 1 micromere formed.

June 27 No. 74. ⊥ 1 : 1.
N 16 cells; no micromeres.
NN 16 cells; 4 micromeres.

June 28 No. 77. ⊥ 8 : 1. Inseminated $11^h\ 50^m$.
N in 2-cell $12^h\ 50^m$; in 4-cell $12^h\ 55^m$; 8-cell $1^h\ 20^m$; 16 cells, 4 micromeres $2^h\ 00^m$.
NN in 2-cell $12^h\ 50^m$; irregular 4-cell $1^h\ 10^m$; later divisions irregular.

No. 80. ⊥ 1 : 1. Inseminated $12^h\ 10^m$.
N 2-cell $1^h\ 10^m$; 4-cell $1^h\ 35^m$; 8-cell $1^h\ 50^m$; formed 3 micromeres
NN amphiaster $1^h\ 10^m$; in 2-cell $1^h\ 35^m$; 4-cell $1^h\ 50^m$; regular 8-cell; formed 1 micromere.

June 29 No. 88. ⊥ 1 : 1. Inseminated $10^h\ 50^m$.
N irregular 3-cell $11^h\ 57^m$.
NN 2-cell $11^h\ 57^m$.
Both 16 cells, no micromeres $1^h\ 20^m$.

No. 89. ⊥ 1 : 1. Inseminated $11^h\ 10^m$.
N 2-cell $12^h\ 00^m$.
NN 2-cell $12^h\ 00^m$.
2 micromeres on both N and NN at $1^h\ 00^m$.

No. 92. ⊥ 2 : 1. Inseminated $11^h\ 25^m$.
Amphiasters, no division $12^h\ 05^m$.
Both 16 cells, no micromeres $1^h\ 40^m$.

No. 94. ⊥ 1 : 1. Inseminated $11^h\ 30^m$.
N 3 micromeres $1^h\ 50^m$.
NN 1 micromere (?) $1^h\ 50^m$, but NN is only in 5-cell stage.

No. 96. ⊥ 1 : 1. Inseminated $11^h\ 36^m$.
N 16 cells, no micromeres $2^h\ 00^m$.
NN one of first two blastomeres failed to divide.

June 30 No. 99. ‖ 1 : 1. Contents stirred in cutting. Inseminated $11^h\ 02^m$.
N 2 micromeres $12^h\ 45^m$.
NN 3 micromeres $12^h\ 47^m$.

No. 104. ‖ 1 : 1. Inseminated $11^h\ 54^m$.
N in 8-cell $1^h\ 20^m$; 4 micromeres, 3 large and 1 small, were formed.
NN in 4-cell $1^h\ 20^m$; 1 micromere formed.

No. 105. ‖ 1 : 1. Inseminated $4^h\ 09^m$.
N regular 4-cell; into 8-cell $6^h\ 17^m$; formed 2 micromeres $7^h\ 10^m$.
NN regular 4-cell; regular 8-cell; formed 2 micromeres $7^h\ 10^m$.

No. 106. ‖ 1 : 1. Inseminated $4^h\ 15^m$.
N regular cleavage to 8-cell; formed 3 micromeres.
NN no cleavage after 2-cell.

No. 107. ‖ 1 : 1. Inseminated $4^h\ 40^m$.
N regular 2-, 4- and 8-cell; 4 micromeres formed $6^h\ 35^m$.
NN regular 2-, 4- and 8-cell; each of the 8 cells divided equally at $6^h\ 42^m$ giving 16 cells; no micromeres.

July 1 No. 108. ‖ 1 : 1. Inseminated $10^h\ 50^m$.
N in 4 cells $12^h\ 02^m$; regular 8-cell, formed 2 micromeres.
NN did not divide.

No. 109. ‖ 2 : 1. Inseminated $11^h\ 10^m$.
N 2-cell $12^h\ 03^m$; 4-cell $12^h\ 15^m$; into 8-cell $12^h\ 34^m$; 4 micromeres and 12 other cells $12^h\ 50^m$.
NN 2-cell $12^h\ 03^m$; 4-cell $12^h\ 15^m$; into 8-cell $12^h\ 42^m$; 4 micromeres and 12 other cells $12^h\ 50^m$.

No. 110. ‖ and horizontal 2 : 1. Inseminated $11^h\ 20^m$.
N 2-cell $12^h\ 05^m$; 4-cell into 8- $12^h\ 25^m$; formed 4 micromeres.
NN into 2-cell $12^h\ 05^m$; 2 micromeres, without question; possibly 4.

July 1 No. 112. ‖ 1 : 1. Inseminated $11^h\ 37^m$.
N in 2-cell $12^h\ 33^m$; regular 4-cell; in 8-cell $1^h\ 45^m$; 2 micromeres and 12 other cells $1^h\ 55^m$.
NN divided irregularly.

No. 113. ‖ 2 : 1. Inseminated $11^h\ 47^m$.
N regular 2-, 4- and 8-cell; 2 micromeres and 8 other cells $2^h\ 10^m$.
NN regular 2-, 4- and 8-cell; 2 micromeres and 8 other cells $2^h\ 10^m$.

July 2 No. 115. ‖ 1.5 : 1. Inseminated $10^h\ 03^m$.
N in 2-cell $10^h\ 52^m$; in 4-cell $11^h\ 11^m$; in 8-cell $11^h\ 33^m$; 3 small and 1 large micromere, 16-cell $11^h\ 58^m$.
NN into 2-cell $10^h\ 52^m$; in 4-cell $11^h\ 15^m$; into 8-cell $11^h\ 35^m$; 3 small and 1 large micromere, 12-cell, $12^h\ 00^m$.

No. 116. ‖ 24 : 1. Inseminated $10^h\ 03^m$.
N in 2-cell $10^h\ 53^m$; in 4-cell $11^h\ 07^m$; in 8-cell $11^h\ 25^m$; amphiasters of next division $11^h\ 30^m$; 4 micromeres formed, 12-cell, $11^h\ 46^m$; upper 4 into 8 giving a 16-cell $11^h\ 47^m$.
NN in 2-cell $10^h\ 53^m$; 4-cell $11^h\ 17^m$; regular 8-cell; each cell divided equally giving 16-cell, no micromeres.

No. 117. ‖ 1 : 2. Inseminated $10^h\ 08^m$.
N in 2-cell $10^h\ 54^m$; 4-cell $11^h\ 18^m$; 8-cell $11^h\ 45^m$; 4 micromeres $12^h\ 05^m$, 16-cell $12^h\ 05^m$.
NN amphiaster $10^h\ 54^m$; in 2-cell $11^h\ 08^m$; in 5-cell (one of first blastomeres failed to divide and other divided twice), $11^h\ 45^m$; each of the four divided again giving a 9-cell stage $12^h\ 15^m$; amphiaster present in the large undivided cell. The 8-celled portion went on developing; the 1-celled portion disintegrated. Did not have chance to follow later details.

No. 118. ‖ 2 : 1. Inseminated $10^h\ 15^m$.
N in 2-cell $11^h\ 05^m$; regular 4- and 8-cell; 3 micromeres in 12-cell stages.
NN in 2-cell $11^h\ 19^m$; regular 4- and 8-cell; 2 micromeres in 16-cell stages.

No. 119. ‖ 1 : 1. Inseminated $10^h\ 34^m$.
N in 2-cell $11^h\ 09^m$; regular 4- and 8-cell; 4 micromeres and 12 other cells $12^h\ 15^m$; 20 cells $12^h\ 30^m$.
NN in 2-cell $11^h\ 19^m$; regular 4- and 8-cell; 4 micromeres and 12 other cells $12^h\ 30^m$.

No. 121. ‖ 2 : 1. Inseminated $10^h\ 49^m$.
N regular 2-, 4- and 8-cell; 4 micromeres and 12 other cells $12^h\ 40^m$.
NN in 8-cell $12^h\ 40^m$; 3 micromeres (2 smaller, 1 larger), and 12 other cells $12^h\ 50^m$.

No. 122. ‖ 1 : 1. Inseminated $1^h\ 50^m$.
N in 3-cell $3^h\ 00^m$; in 6-cell $3^h\ 15^m$; at $3^h\ 30^m$ two of the cells each gave rise to a micromere; two others divided equally, forming a 10-cell stage (fig. 53).
NN irregular cleavage.

Fig. 53

July 3 No. 126. ⊥ 1 : 1. Inseminated $10^h\ 19^m$.
N in 2-cell $11^h\ 25^m$; 4-cell $11^h\ 33^m$; 8-cell $11^h\ 50^m$; 16 cells of about equal size, no micromeres, $12^h\ 15^m$.
NN in 2-cell $11^h\ 25^m$; 4-cell $11^h\ 40^m$; 8-cell $12^h\ 02^m$; 16 cells of about equal size, no micromeres, $12^h\ 27^m$.

July 3 No. 130. ⊥ 1 : 1. Inseminated $10^h\ 53^m$.
N in 8-cell $12^h\ 08^m$; into 16 cells, no micromeres, $12^h\ 38^m$. Active blastula $6^h\ 30^m$.
NN in 4-cell $12^h\ 08^m$; in 8-cell $12^h\ 30^m$; into 16 cells, no micromeres, $12^h\ 40^m$. Active blastula $6^h\ 30^m$.

No. 133. ‖ 1 : 1. Inseminated $11^h\ 12^m$.
N in 8 cells $12^h\ 45^m$; these divided equally into 16, no micromeres, at $1^h\ 02^m$.
NN 2 micromeres and 12 other cells at $1^h\ 12^m$, two of the lower cells not having divided. Both N and NN blastulæ with thick posterior wall; blastocœle of one opaque; of the other clear with 6 free mesenchyme cells, at $6^h\ 15^m$. No trace of neutral red stain that had been used before transfer at $12^h\ 45^m$.

No. 134. ⊥ 1 : 1. Inseminated $11^h\ 12^m$.
Both in 8-cell $12^h\ 46^m$; stained and transferred.
N into 16 cells, no micromeres $1^h\ 00^m$.
NN in 16 cells, no micromeres $1^h\ 15^m$.
Both slowly moving blastulæ, still stained, blastocœle empty at $6^h\ 25^m$.

No. 135. ⊥ 1 : 1. Inseminated $11^h\ 12^m$.
Both in 8-cell $12^h\ 47^m$; stained and transferred.
N 3 smaller micromeres, 1 larger, and 12 other cells $1^h\ 05^m$.
NN to 16 cells, no micromeres; in 32 equal cells, no micromeres, $1^h\ 20^m$. Both blastulæ $6^h\ 35^m$. N stained and inactive; NN active but not rotating.

No. 136. 1 : 1. Partial nuclei; one in each fragment. Inseminated $11^h\ 13^m$.
Both in 8-cell $12^h\ 48^m$. Had developed too far for study of micromeres at $1^h\ 22^m$. One fragment cleaved irregularly; one reached about a 64-cell stage.

July 4 No. 137. Vertical. 1 : 1. Inseminated $10^h\ 25^m$.
N in 2-cell $11^h\ 10^m$; 4-cell $11^h\ 30^m$; into 8- $11^h\ 47^m$; forming 4 micromeres $12^h\ 10^m$; upper cells dividing equally giving a 16-cell stage $12^h\ 10^m$.
NN into 2-cell $11^h\ 16^m$; into 4-cell $11^h\ 40^m$; in 8-cell $12^h\ 00^m$; divided to 16 cells, no micromeres, $12^h\ 17^m$.

No. 140. Horizontal. 1 : 1. Inseminated $1^h\ 50^m$.
N in 2-cell $2^h\ 30^m$; in 4-cell $2^h\ 46^m$; into 8-cell $3^h\ 07^m$; 2 micromeres $3^h\ 27^m$.
NN into 2-cell $2^h\ 40^m$; in 4-cell $3^h\ 00^m$; in 8-cell $3^h\ 25^m$; 2 micromeres $3^h\ 40^m$.

July 5 No. 141. ‖ 1 : 1. Inseminated $10^h\ 30^m$.
N in 2-cell $11^h\ 22^m$; regular 4-cell; regular 8-cell; 4 micromeres $12^h\ 45^m$.
NN 3 cells $11^h\ 52^m$, one of the first two blastomeres having divided; amphiaster in undivided cell; into 5 cells at $12^h\ 06^m$. The larger cell still remaining undivided; no further divisions.

No. 142. ⊥ 1 : 1. Inseminated $10^h\ 44^m$.
N regular 2-, 4- and 8-cell; 4 micromeres $12^h\ 35^m$; 16 cells $1^h\ 10^m$.
NN regular 2-, 4- and 8-cell; 2 micromeres and 14 other cells $12^h\ 35^m$.

No. 143. ‖ 1.5 : 1. Inseminated $10^h\ 49^m$.
N into 2-cell $11^h\ 52^m$; regular 4- and 8-cell; 2 micromeres and 12 other cells $12^h\ 55^m$.
NN regular 8-cell; 1 micromere and 14 other cells; 2 of lower cells did not divide.

July 5 No. 144 ‖ 1 : 1. N regular 8-cell; 3 smaller and 1 larger micromere in 16-cell stage.
NN regular 8-cell; 4 micromeres in the 12-cell stage.

No. 145. ⊥ 1 : 2. Inseminated $11^h\ 30^m$.
N in 8-cell stage 2 blastomeres smaller than others; 4 micromeres at $1^h\ 37^m$, 2 larger and 2 smaller.
NN lost in transferring after regular 2-, 4- and 8-cell.

No. 146. ⊥ 2 : 1. Cut without compression.
N regular 2-, 4- and 8-cell; 16-cell stage with 4 micromeres; these micromeres divided to 8 at $1^h\ 50^m$.
NN regular 2-, 4- and 8-cell; 16-cell with 4 micromeres $1^h\ 45^m$.

No. 147. ⊥ 1 : 1. Inseminated $11^h\ 48^m$.
Both regular cleavage, but developed too far for study of micromeres.

No. 148. ‖ 1 : 1. Inseminated $11^h\ 55^m$.
Both regular cleavage, but developed too far for study of micromeres.

No. 149. ⊥ Inseminated $2^h\ 45^m$.
N into 2-cell $3^h\ 35^m$; 4-cell $4^h\ 05^m$, 2 smaller and 2 larger cells; 8-cell $4^h\ 25^m$, two blastomeres very small; 2 micromeres $4^h\ 58^m$.
NN 2-cell $3^h\ 56^m$; 4-cell $4^h\ 16^m$, 2 smaller and 2 larger cells; 9-cell, 1 micromere $4^h\ 45^m$.

No. 151. ‖ 1 : 1. Cut without compression. Inseminated $2^h\ 55^m$.
N 2-cell $3^h\ 40^m$; 4-cell $3^h\ 57^m$; 8-cell $4^h\ 20^m$; 2 micromeres $4^h\ 30^m$; 2 micromeres and 10 other cells $4^h\ 40^m$; 4 micromeres, 16-cell stage $5^h\ 00^m$.
NN Simultaneous 4-cell $3^h\ 43^m$; 8-cell $4^h\ 20^m$; 4 micromeres, 12-cell stage, $5^h\ 00^m$.

No. 152. ⊥ 1 : 1. Inseminated $2^h\ 55^m$.
N into 2-cell $3^h\ 40^m$; in 4-cell $4^h\ 21^m$; 4 micromeres, 16-cell stage, $5^h\ 05^m$.
NN 2-cell $4^h\ 00^m$; in 4-cell $4^h\ 21^m$; 4 micromeres, 16-cell stage, $5^h\ 07^m$.

No. 153. ⊥ 2 : 1. Cut without compression. Inseminated $3^h\ 03^m$.
N in 2-cell $4^h\ 00^m$; in 4-cell $4^h\ 24^m$; regular 8-cell; 2 smaller and 2 larger micromeres in 12-cell stage $5^h\ 00^m$.
NN in 2-cell $4^h\ 00^m$; in 4-cell $4^h\ 24^m$; regular 8-cell; 2 micromeres and 8 other cells $5^h\ 00^m$.

No. 154. ⊥ 1 : 4. Cut without compression.
N 2-cell $4^h\ 01^m$; regular 4- and 8-cell; 4 micromeres.
NN 2-cell $4^h\ 01^m$; regular 4- and 8-cell; 4 micromeres in 16-cell stage $5^h\ 10^m$.

No. 155. ‖ 1 : 1. Inseminated $3^h\ 08^m$.
N regular cleavage to 8-cell; 2 micromeres and 10 other cells.
NN regular cleavage to 8-cell; 2 micromeres and 10 other cells.

No. 156. Horizontal. 1 : 1. Inseminated $3^h\ 15^m$.
Both 2-cell $4^h\ 02^m$; regular 4- and 8-cell; 4 micromeres on each.

No. 157. ‖ 1 : 1. Inseminated $3^h\ 23^m$.
N 4 micromeres, 16-cell stage, $5^h\ 40^m$.
NN 16 equal cells, no micromeres, $5^h\ 40^m$.

July 6 No. 158. ⊥ 1 : 1. Inseminated $10^h\ 11^m$.
N 4-cell $11^h\ 25^m$; regular 8-cell $12^h\ 00^m$; 3 micromeres and 12 other cells $12^h\ 20^m$.
NN 2-cell $11^h\ 25^m$; 4-cell $12^h\ 00^m$; 2 micromeres (?) and 6 other cells $12^h\ 23^m$. (Interpreted after later studies as an 8-cell stage.)

July 6 No. 159. || 1 : 1. Inseminated 10^h 15^m.
Both 4-cell 11^h 25^m; in 8-cell 11^h 40^m; 2 small cells, micromeres (?), and 6 other cells. [Later observations showed that this was the actual 8-cell stage; 2 of the blastomeres being small in each.] (See No. 158 above.)

No. 160. ⊥ 1 : 1. Inseminated 10^h 22^m.
N 4-cell 11^h 26^m; in 8-cell 11^h 55^m.
NN 2-cell 11^h 26^m; 4-cell 11^h 43^m; both transferred at 11^h 55^m; lost.

No. 161. || 1.5 : 1. Cut without rolling. Inseminated 10^h 22^m.
N 2-cell 11^h 27^m; 4-cell 11^h 42^m; 16 cells, 2 slightly smaller than others, 12^h 25^m; no micromeres.
NN undivided 11^h 42^m; 4 cells, one smaller and transparent, 12^h 25^m; same 12^h 45^m.

No. 163. || 1 : 1. Cut without rolling. Inseminated 10^h 42^m.
N regular 2-, 4- and 8-cell; 3 micromeres and 8 other cells 12^h 52^m.
NN regular 2-, 4- and 8-cell; 1 micromere and 8 other cells 12^h 52^m

No. 164. || 1 : 1. Inseminated 10^h 42^m.
N regular 2-, 4- and 8-cell; 4 micromeres; 16-cell stage.
NN regular 2-, 4- and 8-cell; 4 micromeres; 16-cell stage.

No. 165. ⊥ 1.5 : 1. Compressed in cutting. Inseminated 10^h 50^m.
N regular 2-, 4- and 8-cell. 4 micromeres formed at 1^h 05^m.
NN regular 2-, 4- and 8-cell; 16 cells; could not see micromeres; may have been there.

No. 167. ⊥ 1 : 1. Egg elongated. Cut across long axis. Inseminated 11^h 04^m.
N regular 2-, 4- and 8-cell; 1 micromere and 11 other cells.
NN regular 2-, 4- and 8-cell; had developed beyond 16-cell stage when next examined; too late for determination of micromeres.

No. 169. ⊥ 1 : 1. Inseminated 11^h 12^m.
N regular 2-, 4- and 8-cell; 3 micromeres; 6 macromeres; 8 mesomeres; 17 cells.
NN regular 2-, 4- and 8-cell; divided to 16 cells 1^h 25^m; no micromeres.

No. 170. ⊥ 1 : 1. Inseminated 11^h 12^m.
N regular 2-, 4- and 8-cell; 4 micromeres 1^h 25^m.
NN cytolized.

No. 171. || 1 : 1. Inseminated 2^h 00^m.
N 2-cell 2^h 50^m; 4-cell 3^h 22^m; into 8-cell 3^h 35^m; 2 micromeres and 8 other cells 4^h 10^m to 4^h 20^m; 2 micromeres and 12 other cells 4^h 25^m. Blastomeres much flattened against each other.
NN no record.

No. 172. ⊥ 1 : 1. Inseminated 2^h 20^m.
N in 2-cell 3^h 04^m; 4-cell 3^h 25^m; 8-cell 3^h 50^m; later divisions could not be followed.
NN into 2-cell 3^h 15^m; 4-cell 3^h 45^m; 8-cell 4^h 10^m; later divisions could not be followed. The blastomeres in these fragments were much flattened against each other.

No. 173. ⊥ 1 : 1. Inseminated 2^h 30^m.
N 2-cell 3^h 16^m; regular 4- and 8-cell; 2 micromeres and 12 other cells 4^h 20^m; still only 2 micromeres 4^h 30^m.
NN regular 2-, 4- and 8-cell; 2 micromeres and 10 other cells 4^h 35^m.
The blastomeres in these fragments were much flattened against each other.

July 7 No. 174. ‖ 1 : 1.5. Inseminated $10^h\ 15^m$.
N regular 2-cell; in 4-cell $11^h\ 22^m$; 8-cell $11^h\ 35^m$; 3 micromeres, 3 macromeres, lower cell that divided equally to two cells, and 8 mesomeres, giving 16 cells at $12^h\ 10^m$.
NN regular 2-cell; 3-cell at $11^h\ 22^m$, division being delayed in one blastomere; regular 4-cell; 8-cell $11^h\ 45^m$. Divided to 16 equal cells $11^h\ 55^m$, no micromeres; each of these cells divided equally in the next division.

No. 175. ⊥ 1 : 1. Inseminated $10^h\ 15^m$.
N 2-cell $11^h\ 15^m$; 4-cell $11^h\ 24^m$; irregular 8-cell, 2 micromeres (?); 2 macromeres and 4 mesomeres $11^h\ 55^m$. (Explanation same as Nos. 158 and 159.)
NN 2-cell $11^h\ 15^m$; 4-cell $11^h\ 25^m$; 2 micromeres and 8 other cells $11^h\ 50^m$, division having failed in 2 of the lower cells.

No. 176. ⊥ 1 : 1. Inseminated $10^h\ 20^m$.
N regular 2-, 4- and 8-cell; 2 micromeres and 10 other cells, two of the lower cells having divided equally.
NN regular 2-, 4- and 8-cell; micromeres not determined.

No. 178. ‖ 1.5 : 1. Inseminated $10^h\ 40^m$.
N 2-cell $11^h\ 30^m$; regular 4-cell; 8-cell $12^h\ 00^m$; 2 micromeres $12^h\ 40^m$.
NN 2-cell $12^h\ 00^m$; 4-cell $12^h\ 20^m$; 8-cell $12^h\ 40^m$; 2 micromeres and 10 other cells, 2 of the lower cells having divided equally $1^h\ 00^m$.

No. 180. ⊥ 1.5 : 1. Inseminated $10^h\ 50^m$.
N 2-cell $11^h\ 26^m$; regular 4-cell; 8-cell $12^h\ 30^m$; one of the lower cells divided to 2 equal cells, and one formed a micromere $12^h\ 35^m$, giving a 10-cell stage.
NN undivided at $11^h\ 40^m$; in 4-cell $12^h\ 40^m$; 8-cell $1^h\ 00^m$; not followed further.

No. 181. ⊥ 1 : 1. Inseminated $11^h\ 04^m$.
N amphiaster $11^h\ 42^m$; in 4-cell $12^h\ 20^m$; regular 8-cell; 2 micromeres, 6 macromeres (4 of these resulting from the equal division of 2 lower cells that did not form micromeres), and 8 mesomeres, giving a 16-cell stage, $1^h\ 12^m$.
NN 2-cell $12^h\ 20^m$; regular 4- and 8-cell; 4 micromeres, 16-cell stage $1^h\ 45^m$.

No. 182. ⊥ 1 : 1. Inseminated $11^h\ 17^m$.
N regular 2-cell; 4-cell $12^h\ 20^m$; regular 8-cell; overstained.
NN regular 2-cell $12^h\ 20^m$; 4- and 8-cell; overstained.

No. 183. ⊥ 1 : 1. Inseminated $2^h\ 06^m$.
N regular 2-cell; 4-cell $3^h\ 10^m$; 8-cell $3^h\ 25^m$; 2 micromeres and 14 other cells, two of the lower cells forming micromeres and two dividing equally.
NN 2-cell $3^h\ 10^m$; regular 4-cell; 8-cell $3^h\ 25^m$; 2 micromeres and 14 other cells, identical with N.

No. 184. ⊥ 1 : 1. Inseminated $2^h\ 06^m$.
N 2-cell $3^h\ 11^m$; 4-cell $3^h\ 22^m$; 8-cell $3^h\ 40^m$; seemed to have 2 micromeres and 14 other cells $4^h\ 45^m$.
NN amphiaster $3^h\ 11^m$; 2-cell $3^h\ 22^m$; 4-cell $3^h\ 40^m$; regular 8-cell; seemed to have 2 micromeres and 14 other cells $4^h\ 45^m$.

No. 185. ‖ 1 : 1. Inseminated $2^h\ 08^m$.
N 3-cell $3^h\ 12^m$; one of first two blastomeres slow in dividing; 4-cell $3^h\ 22^m$; 8-cell $3^h\ 30^m$; lost during division probably to 16 equal cells $3^h\ 55^m$.
NN 2-cell $3^h\ 12^m$; 4-cell $3^h\ 22^m$; regular 8-cell; 4 micromeres (3 small and 1 large), 4 macromeres, 8 mesomeres $4^h\ 20^m$.

July 7 No. 187. ⊥ 1 : 1.5. Inseminated $2^h\ 15^m$.
N in 2-cell with amphiasters of second division $3^h\ 15^m$; regular 4- and 8-cell; 1 micromere and 15 other cells $3^h\ 50^m$, one of the lower cells having formed a micromere, the other three dividing equally.
NN 2-cell $3^h\ 15^m$; regular 4- and 8-cell; 3 micromeres and 13 other cells $3^h\ 55^m$, three of the lower cells each formed a micromere and one divided equally. The three micromeres were each different in size.

No. 188. ⊥ 1 : 1. Inseminated $2^h\ 34^m$.
N 2-cell $3^h\ 16^m$; 4-cell $3^h\ 55^m$; 8-cell $3^h\ 58^m$; 16 equal cells $4^h\ 40^m$.
NN not divided $3^h\ 16^m$; not divided $3^h\ 55^m$; did not divide.

No. 189. ‖ 1 : 1. Inseminated $2^h\ 34^m$.
N 2-cell $3^h\ 17^m$; regular 4- and 8-cell; 1 micromere and 15 other cells in the 16-cell stage (one of the lower cells formed a micromere and then divided equally).
NN amphiaster $3^h\ 17^m$; regular 2-, 4- and 8-cell, 3 micromeres (one smaller and two larger), present in the 16-cell stage, three of the lower cells having formed micromeres and one having divided equally.

No. 191. ‖ 1 : 1. Inseminated $2^h\ 51^m$.
N regular 2- and 4-cell. One of the blastomeres was delayed in its next division giving a 7-cell stage; one of the three lower cells then formed a micromere, giving an 8-cell stage consisting of one undivided 4-cell stage blastomere, one micromere, one macromere, two lower cells and three upper cells. The three upper cells then divided equally to form 6 mesomeres, giving an 11-cell stage. The next division was an equal division of each of these 11 cells giving a 22-cell stage. These were an upper and a lower cell from the ¼ blastomere, 12 cells as a result of two divisions of each of the 3 mesomeres, 4 cells from the equal division of two lower cells, 2 micromeres from the division of the original micromere, and 2 cells from the division of the macromere.

July 9 No. 192. Horizontal, 2 : 1. Inseminated $9^h\ 57^m$.
N regular 2-cell; 4-cell $11^h\ 07^m$; 8-cell $11^h\ 19^m$; micromeres were formed by 2 lower cells, the other 2 dividing equally $11^h\ 40^m$; the 4 upper cells (mesomeres) then divided to 8 giving a 16-cell stage.
NN 2-cell $11^h\ 07^m$; 4-cell $11^h\ 19^m$; 8-cell $11^h\ 44^m$, four of the cells being larger, and 4 smaller and of two sizes, (as in fig. 37). One of the smallest cells formed a micromere $12^h\ 00^m$; the other of the smallest cells formed a micromere at $12^h\ 01^m$; both of the two remaining smaller cells formed micromeres at $12^h\ 02^m$. The four upper cells then divided equally giving a 16-cell stage, $12^h\ 07^m$ to $12^h\ 10^m$.

No. 193. Horizontal. 1 : 2. Inseminated $10^h\ 18^m$.
N 2-cell $11^h\ 08^m$; regular 4-cell; 8-cell $11^h\ 53^m$; 2 micromeres, one smaller, one larger, $12^h\ 05^m$.
NN amphiaster $11^h\ 08^m$; regular 4-cell $12^h\ 05^m$; 8-cell, two of blastomeres being smaller than the others $1^h\ 10^m$. Not followed further.

No. 194. Horizontal. 1 : 8. Inseminated $10^h\ 25^m$.
N cytolized.
NN regular 2-, 4- and 8-cell. 3 micromeres, one lower cell divided equally to 2; 3 macromeres, 8 mesomeres, giving a 16-cell stage $12^h\ 15^m$.

July 9 No. 195. Horizontal. 1 : 1. Inseminated $10^h\ 50^m$.
N regular 2-, 4- and 8-cell; 3 micromeres, 3 macromeres, one lower cell that had divided equally, and 8 mesomeres, forming a 16-cell stage $1^h\ 10^m$.
NN regular 2-, 4- and 8-cell; 2 micromeres, in a 10-cell stage $1^h\ 10^m$.

No. 197. Horizontal. 30 : 1. Nucleus in vegetal fragment. Inseminated $2^h\ 26^m$.
N 2-cell $3^h\ 00^m$; 4-cell $3^h\ 20^m$; 8-cell $3^h\ 45^m$.
NN amphiaster $3^h\ 00^m$; 2-cell $3^h\ 16^m$; regular 4- and 8-cell.
After transfer to slide both were very compact and the later divisions could not be followed.

July 10 No. 198. Horizontal. 1 : 2. Nucleus in vegetal fragment. Inseminated $10^h\ 10^m$.
N 2-cell $11^h\ 00^m$; 4-cell $11^h\ 25^m$; 2 of these 4 cells divided earlier than the remaining two $11^h\ 35^m$; 8-cell $12^h\ 00^m$. Did not develop further after transfer.
NN cytolized.

No. 199. Horizontal. 1 : 6. Nucleus in animal fragment. Inseminated $10^h\ 50^m$.
N 2-cell $11^h\ 36^m$; 4-cell $12^h\ 05^m$; 8-cells $12^h\ 30^m$; 12-cells, no micromeres, $12^h\ 50^m$; 16-cells, no micromeres, $1^h\ 00^m$.
NN 2-cell $12^h\ 05^m$; regular 4-cell; 8-cell $12^h\ 50^m$; 4 micromeres formed and the 4 mesomeres divided to 8; giving a 16-cell stage at $1^h\ 00^m$.

No. 200. Horizontal. 2 : 1. Nucleus in vegetal fragment. Inseminated $11^h\ 17^m$.
N 2-cell $12^h\ 06^m$; regular 4- and 8-cell; 2 micromeres, 4 macromeres and 4 mesomeres at $1^h\ 10^m$.
NN into 2 cells $12^h\ 06^m$; regular 4- and 8-cell; 2 micromeres and 10 other cells, forming a 12-cell stage $1^h\ 15^m$.
The observations on these two fragments were not thoroughly satisfactory.

No. 201. Horizontal. 1 : 1. Nucleus in animal fragment. Inseminated $11^h\ 26^m$.
N regular 2-cell; 4-cell $12^h\ 40^m$; 8-cell $1^h\ 05^m$; 16 cells, no micromeres $1^h\ 26^m$.
NN 2-cell $12^h\ 40^m$; regular 4-cell; 8-cell $1^h\ 25^m$; 4 micromeres, 4 macromeres, 8 mesomeres $1^h\ 30^m$, the micromeres all different in size.

No. 202. Horizontal. 1 : 1. Nucleus in vegetal fragment. Inseminated $11^h\ 38^m$.
N regular 2-cell; 4-cell $12^h\ 41^m$; regular 8-cell; 4 micromeres, 8 macromeres, 8 mesomeres, a 20-cell stage, $1^h\ 42^m$.
NN 2-cell $12^h\ 41^m$; regular 4- and 8-cell; 12 cells, no micromeres $1^h\ 38^m$.

No. 203. Horizontal. 1 : 15. Nucleus in animal fragment. Inseminated $2^h\ 23^m$.
N 2-cell $3^h\ 20^m$; 4-cell $3^h\ 28^m$; into 8-cell $3^h\ 30^m$; two of the 8 cells were much smaller than the remaining six; 12-cell stage, no micromeres at $3^h\ 55^m$; cell outlines no longer visible at $4^h\ 00^m$.
NN not divided $3^h\ 20^m$; no further record.

July 11 No. 204. Horizontal. 1 : 30. Nucleus in animal fragment.
N 2-cell 1^h 20^m; 4-cell 1^h 47^m; 8-cell 2^h 05^m; 16 cells, no micromeres 2^h 23^m.
NN formed fertilization membrane, no further record.

No. 205. Horizontal. 1 : 1. Nucleus in vegetal fragment. Inseminated 1^h 00^m.
N 2-cell 2^h 10^m; 4-cell 2^h 38^m; 8-cell 2^h 55^m. Too compact for study after transfer.
NN amphiaster 2^h 10^m; did not develop.

No. 206. Horizontal. 1.5 : 1 Nucleus in vegetal fragment. Inseminated 1^h 10^m.
N 2-cell 2^h 10^m; regular 4-cell; 8-cell 2^h 41^m, 4 cells being larger than remaining 4; 2 of the larger cells divided equally 2^h 58^m; 2 smaller and 1 larger cell formed micromeres 2^h 59^m; all other cells divided equally at 3^h 00^m, giving 16-cell stage.
NN did not divide.

No. 209. Horizontal. 1 : 2. Nucleus in animal fragment. Inseminated 1^h 57^m.
N regular 2-cell; 4-cell 3^h 20^m; regular 8-cell; 12-cell, no micromeres, 3^h 45^m. Did not develop further.
NN in 4-cell 3^h 20^m; 8-cell 4^h 15^m; into 12-cell 4 micromeres 4^h 30^m.

July 12 No. 210. Horizontal. 2 : 1. Nucleus in vegetal fragment. Inseminated 10^h 12^m.
N 2-cell 10^h 55^m; 4-cell 11^h 24^m; 8-cell 11^h 46^m; 3 micromeres, 4 macromeres, 8 mesomeres 12^h 17^m.
NN amphiaster 11^h 05^m; 2-cell 11^h 17^m; 4-cell 11^h 40^m; 8-cell 12^h 05^m; 2 micromeres, 4 macromeres, 8 mesomeres.

No. 211. Horizontal. 1 : 4. Nucleus in animal fragment. Inseminated 10^h 20^m.
N 2-cell 11^h 10^m; 4-cell 11^h 21^m; 8-cell 11^h 45^m; 3 micromeres and 10 other cells 12^h 10^m.
NN amphiaster 11^h 10^m; 2-cell 11^h 21^m; 4-cell 11^h 45^m; 6-cell 11^h 50^m; further cleavage irregular, 12^h 21^m.

No. 213. Horizontal. 2 : 1. Nucleus in vegetal fragment. Inseminated 10^h 55^m.
N regular 2-, 4- and 8-cell; 4 micromeres, 4 macromeres, 8 mesomeres, 12^h 50^m.
NN irregular cleavage.

No. 214. Horizontal. 1.5 : 1. Nucleus in vegetal fragment. Inseminated 11^h 14^m.
N regular 2-, 4- and 8-cell; 4 micromeres, 4 macromeres, 8 mesomeres 12^h 55^m; 4 macromeres divided to 8, 12^h 58^m.
NN regular 2-, 4- and 8-cell; 3 micromeres, 4 macromeres, 8 mesomeres, 1^h 10^m.

July 13 No. 215. Horizontal. 8 : 1. Nucleus in vegetal fragment. Inseminated 10^h 12^m.
N 2-cell 10^h 57^m; 4-cell 11^h 17^m; 8-cell 11^h 36^m; 4 micromeres formed 11^h 57^m; 4 mesomeres divided to 8 at 11^h 57^m; 16-cell stage.
NN not divided 11^h 17^m; 2-cell 11^h 26^m; into 4-cell 11^h 48^m; into 8-cell 12^h 32^m; 4 of the 8 cells divided equally 12^h 40^m; the other 4 seem to have gone back to 2 cells; these 2 seem to have fused at 12^h 50^m, giving the appearance of a 9-cell stage. In later development the cleaving fragment became a hollow sphere with a large fragment attached to it.

July 13 No. 216. Horizontal. 3 : 1. Nucleus in vegetal fragment. Inseminated $10^h\ 37^m$.

N 2-cell $11^h\ 18^m$; 4-cell $11^h\ 39^m$; 8-cell $12^h\ 10^m$; 3 micromeres formed at $12^h\ 22^m$; 12 other cells making a 15-cell stage.

NN protrusion $11^h\ 15^m$; no further development.

No. 217. Horizontal. 1 : 4. Nucleus in animal fragment. Inseminated $11^h\ 06^m$.

N in oblique 2-cell $11^h\ 52^m$; 4-cell into 8- at $1^h\ 00^m$; five of these divided equally $1^h\ 02^m$ to $1^h\ 05^m$; two others formed micromeres; one did not divide.

The result of this cleavage was a 15-cell stage with 2 micromeres.

NN regular 2- and 4-cell; into 8-cell at $1^h\ 00^m$; 2 micromeres formed as the mesomeres were dividing, giving a 14-cell stage.

July 14 No. 220. Horizontal. 3 : 1. Nucleus in vegetal fragment. (Eggs cooled.) Inseminated $12^h\ 55^m$.

N into 2-cell $1^h\ 34^m$; 4-cell $2^h\ 02^m$; into 8-cell $2^h\ 22^m$. Two of the upper cells divided equally at $2^h\ 45^m$, another at $2^h\ 46^m$, another at $2^h\ 47^m$; each of the 4 lower cells formed a micromere, 2 larger than the other 2, at $2^h\ 46^m$.

NN into 2-cell $2^h\ 08^m$; in 4-cell $3^h\ 05^m$; 6-cell $3^h\ 15^m$; 10-cell $3^h\ 26^m$ to $3^h\ 29^m$; later divisions irregular.

July 16 No. 221. Horizontal. 1 : 2. Nucleus in animal fragment. Inseminated $11^h\ 30^m$.

N regular 2-, 4- and 8-cell; 16 cells without micromeres $1^h\ 30^m$. July 17, $8^h\ 00^m$ a.m. rotating stereoblastula; no pigmented cells.

NN regular 2-, 4- and 8-cell; 12 cells without micromeres $1^h\ 30^m$. July 17, $8^h\ 00^m$ a.m. rotating stereoblastula; many pigmented cells.

No. 223. Horizontal. 1 : 2.5. Nucleus in animal frament. Inseminated $1^h\ 52^m$.

N into 2-cell $2^h\ 36^m$; 4-cell $3^h\ 04^m$; 8 cells $3^h\ 22^m$, 3 small and of same size, 1 slightly larger and 4 large cells (fig. 28). At $3^h\ 45^m$ one of the smallest cells formed a micromere and 3 of the large cells divided equally; at $3^h\ 50^m$ another of the smallest cells, and at $3^h\ 51^m$ the third of the smallest cells, formed a micromere. At $3^h\ 55^m$ the slightly larger cell of the group of small cells divided equally, these 2 cells lying close to the 3 micromeres. The previously undivided cell of the original group of 4 large cells divided at $3^h\ 52^m$. The fragment was transferred back to sea-water at $4^h\ 10^m$. (The cleavage pattern of this fragment suggested localization of endoderm-forming substance.) July 17, $8^h\ 00^m$ a.m., gastrula with small archenteron; thickened and distorted skeletal rods.

NN not divided $3^h\ 00^m$; irregular 2-cell $3^h\ 04^m$; into 3 cells $3^h\ 25^m$, division lagging in one blastomere; this slow cell did not develop well; the remaining cells (one-half of the fragment), continued their cleavage. No evidence on micromeres could be obtained.

No. 224. Horizontal. 1 : 4. Nucleus in animal fragment. Inseminated $2^h\ 46^m$.

N regular 2- and 4-cell; in 8-cell $4^h\ 21^m$; 4 of the 8 cells divided equally at $4^h\ 40^m$; the other 4 divided equally at $4^h\ 45^m$.

NN no development.

July 17 No. 225. Horizontal. 20 : 1. The non-nucleated fragment was a small piece containing the material surrounding the animal pole. Inseminated 10^h 05^m.

N regular 2-cell; 4-cell 11^h 00^m; 8-cell 11^h 25^m; 2 micromeres formed at 11^h 37^m, the other 2 lower cells dividing equally at the same time, giving a 12-cell stage. The upper 4 had not divided at 11^h 45^m.

NN did not form a fertilization membrane; did not develop.

No. 226. Horizontal. 1 : 8. Nucleus in animal fragment. Inseminated 10^h 12^m

N 2-cell 11^h 05^m; regular 4-cell; 8-cell 11^h 45^m; 2 micromeres formed, other 2 lower cells divided equally, giving a 12-cell stage.

NN in 2-cell 11^h 45^m; at 12^h 17^m one of the first 2 blastomeres had stopped dividing; the other was in 4 cells. (These 4 cells therefore represented half an 8-cell stage.) Three of these cells, divided equally at 12^h 30^m; the fourth cell formed a micromere at 12^h 34^m. The previously undivided blastomere of the 2-cell stage was dividing at 12^h 36^m. Fragment lost in retransfer.

No. 227. Horizontal. 2 : 1. Nucleus close to vegetal pole. Inseminated 10^h 17^m.

N 2-cell 11^h 06^m; regular 4-cell; 8-cell 12^h 00^m; 16-equal cells 12^h 10^m. Gave rise to a perfect active blastula, no mesenchyme in blastocœle at 6^h 00^m; 4 mesenchyme cells in blastocœle at 6^h 45^m.

NN not divided 11^h 06^m; not divided 12^h 00^m.

No. 228. Horizontal. 4 : 1. The non-nucleated fragment contained the material of the region surrounding the animal pole. Inseminated 10^h 27^m.

N 2-cell 11^h 07^m; regular 4- and 8-cell; formed 3 micromeres (2 very small and 1 of normal size); 1 lower cell divided equally; in a 20-cell stage at 12^h 40^m.

NN did not develop.

No. 229. Horizontal. 16 : 1. The non-nucleated fragment contained the region of the animal pole. Inseminated 10^h 27^m.

N in 2-cell 11^h 25^m; regular 4-cell; in 8-cell 12^h 05^m; 2 micromeres formed at 12^h 10^m, one lower cell divided slightly unequally, one divided equally; the 4 mesomeres divided equally at 12^h 11^m, giving a 16-cell stage. Blastula with 16 mesenchyme cells at 5^h 40^m, a perfect larva. July 18, 10^h 00^m a.m., a poorly developed pluteus without arms; œsophagus, stomach and intestine well formed; body skeleton feebly developed, but present on right and left sides of body.

NN in 2-cell 11^h 25^m; regular 4-cell; in 8-cell 12^h 10^m; 16 equal cells, no micromeres 12^h 20^m; a normal, active blastula with 4 mesenchyme cells in blastocœle at 5^h 40^m. July 18, 10^h 00^m a.m., a fine active blastula with mesenchyme. No evidence of gastrulation. July 19, 9^h 00^m a.m. larva still active; no gastrulation; one small triradiate skeletal spicule present.

No. 230. Horizontal. 1 : 1.2. Nucleus in animal fragment. Inseminated 10^h 35^m.

N regular 2- and 4-cell; 8-cell; 16-cell; 20-cell, no micromeres, 12^h 48^m. Mesenchyme cells beginning to migrate from pos-

July 17 No. 230 (cont.).
terior wall of blastula 6^h 25^m; these cells are visible in the wall as well as just entering the blastocœle; a fine blastula. At 6^h 30^m 4 mesenchyme cells in the blastocœle. July 18, 9^h 00^m a.m. gastrula, only 1 skeletal spicule, but 2 groups of mesenchyme cells are symmetrically placed; not quite normal. July 19, pluteus without arms; good body skeleton.
NN regular 2- and 4-cell; 8-cell 12^h 30^m; divided to 16 equal cells, no micromeres. At 6^h 35^m a blastula whose blastocœle was filled completely with cells. July 18, 9^h 00^m a.m., a gastrula with skeletal rods and pigmented cells in the gastrocœle; not quite normal.

No. 231. Horizontal. 1 : 1.3. Nucleus in animal fragment. Inseminated 10^h 45^m.
N regular 2-cell; 4-cell 12^h 00^m; 32 cells, no micromeres, 1^h 04^m; blastula with thickened posterior wall, no mesenchyme cells in blastocœle, 6^h 05^m. July 18, 9^h 00^m a.m. good blastula; no mesenchyme, simply a hollow sphere.
NN 2-cell 12^h 00^m; regular 4- and 8-cell; 2 lower cells formed micromeres, 2 divided equally; the micromeres divided, each to 2 at 1^h 00^m; fragment then in 28-cell stage. An active blastula, many cells in blastocœle at 6^h 05^m. July 18, 9^h 00^m a.m. a gastrula, abnormal in that the gastrocœle is too compactly filled with mesenchyme (?) cells. No skeletal spicules evident, July 19, 3 triradiate spicules present. Did not develop beyond gastrula stage.

No. 232. Horizontal. 49 : 1. The non-nucleated fragment contained the region of the animal pole. The plane of section was just above the nucleus. Inseminated 11^h 01^m.
N poor membrane; very irregular cleavage.
NN no membrane; no cleavage.

No. 233. Horizontal. 1 : 34. Nucleus in the very small animal polar fragment. Inseminated 11^h 20^m. Both formed membranes.
N no development.
NN regular 2- and 4-cell; in 8-cell 12^h 52^m; 2 lower cells formed micromeres, 2 divided equally and the 4 upper cells divided to 8 making a 16-cell stage.

July 18 No. 235. Horizontal. 1.5 : 1. Nucleus in animal fragment. Inseminated 11^h 26^m.
N regular 2- and 4-cell; in 8-cell stage 4 cells were distinctly larger than other 4; larger 4 divided equally 1^h 05^m; 3 of the smaller 4 formed micromeres 1^h 12^m; fourth cell not divided, giving a 15-cell stage.
NN divided to 3 cells; in 6 cells at 1^h 26^m; in 11 cells at 1^h 45^m, one a micromere, formed at 1^h 35^m.

No. 237. Horizontal. 1 : 10. Nucleus in animal fragment. Inseminated 12^h 55^m.
N regular 2-cell; 4-cell 2^h 10^m; in 8-cell 2^h 40^m; 16 cells, no micromeres 3^h 05^m. July 19, dead.
NN 2-cell 2^h 10^m; 4-cell 2^h 25^m; 8-cell 2^h 46^m; 4 micromeres formed 3^h 05^m (fig. 54); 4 mesomeres to 8, 3^h 06^m; July 19, flattened gastrula.

Fig. 54

July 18 No. 238. Horizontal. 3 : 1. Non-nucleated fragment is part of the vegetal hemisphere. Inseminated 1^h 21^m.

N 2-cell 2^h 17^m; regular 4-cell; 8-cell (4 large and 4 small cells), 3^h 20^m. The 4 small cells formed micromeres at 3^h 40^m; the 4 large cells divided equally at 3^h 41^m.

NN regular 2-cell; one of the blastomeres divided at 3^h 30^m; these divided again, no further development.

No. 239. Horizontal. 4 : 1. Nucleus in vegetal fragment. Inseminated 1^h 21^m.

N in 2-cell 2^h 18^m; developed well but there was no time for its study.

NN did not divide.

No. 240. Horizontal. 1 : 4. Nucleus in animal fragment. Inseminated 1^h 27^m.

Fig. 55

N in 2-cell 2^h 20^m; developed to 16 equal cells.

NN amphiaster 2^h 20^m; 1 of the first 2 blastomeres failed to continue cleaving; the other divided to 2 and to 4 cells; 2 of the 4 cells formed micromeres (true half cleavage) (fig. 55). July 19, a stereoblastula.

July 19 No. 242. Horizontal(?). 4 : 1. The non-nucleated fragment was part of the vegetal hemisphere. Inseminated 9^h 40^m.

N 2-cell 10^h 27^m; 4-cell 10^h 50^m; 8-cell 11^h 06^m. In this fragment the second cleavage was parallel to the cut surface (fig. 30). The 8-cell stage consisted of 4 large and 4 small cells (fig. 31); micromeres were formed one each by 2 of the large and 2 of the small cells 11^h 28^m (fig. 8), giving a 12-cell stage. The 4 cells on the opposite side of the fragment then divided to 8, giving a 16-cell stage.

NN 2-cell 10^h 50; in 4-cell 11^h 00^m; 8-cell in which one group of four is small; 16-cell of 4 micromeres, 4 macromeres and 8 mesomeres, the mesomeres being very small, smaller than the micromeres.

No. 246. ⊥ 2 : 1. Inseminated 10^h 08^m.

N 16-cell consisted of 4 micromeres, 4 macromeres and 8 mesomeres.

NN no record.

No. 247. Horizontal. 1 : 36. Nucleus was in very small vegetal polar fragment. Inseminated 10^h 17^m. Both fragments formed membranes.

N did not develop.

NN regular 2- and 4-cell; in 8-cell 12^h 13^m; formed 4 micromeres.

Nos. 248, 249 and 250. Inseminated at 10^h 35^m, 10^h 35^m and 10^h 45^m respectively, all divided well, but were not studied because of lack of time.

No. 251. Horizontal. 1 : 24. Nucleus in small vegetal polar fragment. Inseminated 11^h 22^m.

N regular 2- and 4-cell; in 8-cell at 12^h 47^m; 4 of the cells being slightly smaller than the other 4. Simultaneous equal division of each cell, giving 16 cells, no micromeres, at 1^h 07^m; in 28-cell 1^h 30^m; in 32-cell 1^h 35^m.

NN Cleavage very irregular.

July 21 No. 252. Horizontal. 30 : 1. Non-nucleated fragment contained the material surrounding the vegetal pole. Inseminated $10^h\ 26^m$.

N in 2-cell $11^h\ 07^m$; 4-cell $11^h\ 28^m$; 8-cell $11^h\ 44^m$; 2 of these cells divided, slightly unequally at $12^h\ 02^m$, 2 formed micromeres, and the remaining 4 divided equally, at $12^h\ 03^m$, giving a 16-cell stage with 2 micromeres. July 22, $8^h\ 30^m$ a.m., gastrula with pigment in some of the mesenchyme cells and with skeletal spicules. More than the normal number of mesenchyme cells.

NN in 2-cell $11^h\ 07^m$; in 4-cell $11^h\ 41^m$; in 8-cell $12^h\ 10^m$, 4 cells being slightly larger than the other 4; all of the larger divided before the smaller, slightly unequally, at $12^h\ 19^m$; the 4 smaller cells divided equally at $12^h\ 20^m$, giving a 16-cell stage.

July 22, $8^h\ 30^m$ a.m. disintegrated. The material contained in the non-nucleated fragment was just about that of the "micromere field" of the entire egg. The slightly unequal division at $12^h\ 19^m$ might be interpreted as the formation of micromeres; these cells were not as small, however, as those formed by the division at $12^h\ 20^m$; interpretable as a formation of mesomeres.

No. 253. Horizontal. 10 : 1. Nucleus close to animal pole. Inseminated $10^h\ 50^m$.

N 2-cell $11^h\ 30^m$; 4-cell $11^h\ 50^m$; 8-cell $12^h\ 12^m$; 4 micromeres formed $12^h\ 32^m$ to $12^h\ 35^m$, 2 of them smaller and 2 larger. July 22, $9^h\ 00^m$ a.m. perfect gastrula. July 23, $8^h\ 00^m$ a.m. pluteus without arms.

NN amphiaster $11^h\ 30^m$; 2-cell $11^h\ 50^m$; 4-cell $12^h\ 09^m$; 2 of the 4 cells then divided, giving a 6-cell stage; one of the undivided cells then protruded a small clear, rounded fragment, apparently no nuclear division occurring. The cleavage was not studied further. July 22, $9^h\ 00^m$ a.m., good, normal gastrula. July 23, disintegrated.

No. 254. Horizontal. 1 : 1.1. The nucleus was situated slightly below the equatorial plane. Inseminated $11^h\ 12^m$.

N regular 2-cell; 4-cell $12^h\ 15^m$; in 8-cell $12^h\ 45^m$; from 8 to 16 cells, 4 cells dividing slightly earlier than the other 4, $12^h\ 50^m$; no micromeres.

July 22, $8^h\ 00^m$ a.m. perfect gastrula, flattened in oral and anal areas; skeleton normal. July 23, $8^h\ 00^m$ a.m., pluteus, imperfect in that left arm was lacking.

NN regular 2-cell; 4-cell $12^h\ 15^m$; 8-cell $12^h\ 50^m$; 3 of the lower cells formed micromeres and one divided equally, $12^h\ 51^m$; the 4 mesomeres divided equally at $12^h\ 52^m$. The mesomeres, after this division, were of the same size as the macromeres. Early blastula $5^h\ 00^m$. No later record.

No. 255. Vertical. 1 : 1. Inseminated $2^h\ 06^m$.

N did not develop.

NN into 2-cell $3^h\ 00^m$; regular 4-cell; into 8-cell $3^h\ 45^m$; one blastomere very small, about half the size of the others.

No. 256. Vertical. 1 : 1. Inseminated $2^h\ 21^m$.

The amphiaster formed in the short axis of both fragments.

N the centers rotated, the cell remaining elongated, division occurring across the long axis of the cell. In 4-cell $3^h\ 06^m$; 8-cell $3^h\ 35^m$. No further record.

NN 2-cell $3^h\ 40^m$. No further record.

July 21 No. 257. Vertical. 1 : 1. Inseminated $2^h\ 21^m$.
In both fragments the amphiaster appeared in the shorter axis of the cell. The cell changed form around it.
N 2-cell $3^h\ 07^m$; 4-cell $3^h\ 30^m$; into 8-cell $3^h\ 50^m$, 2 of the cells being distinctly smaller than the others. Three smaller and one larger micromere formed at $4^h\ 15^m$, the 4 mesomeres dividing to 8 simultaneously, giving a 16-cell stage.
NN in 2-cell $3^h\ 10^m$; 4-cell $3^h\ 35^m$; into 8-cell $3^h\ 52^m$, one cell being distinctly smaller than the others; 4 micromeres and 12 other cells at $4^h\ 21^m$.

No. 258. Vertical. 1 : 1. Inseminated $2^h\ 41^m$.
N 2-cell $3^h\ 25^m$; regular 4-cell; 8-cell $4^h\ 10^m$; 16-cell with 4 micromeres $4^h\ 35^m$.
NN amphiaster $3^h\ 25^m$; in 4-cell $4^h\ 10^m$; in 8-cell, 2 cells smaller than others. One of the smaller cells formed a micromere at $4^h\ 37^m$; the other at $4^h\ 40^m$; the 4 mesomeres divided to 8 at $4^h\ 36^m$, giving a 14-cell stage. July 22, distinguishing marks on the two dishes obliterated. One larva had disintegrated; the other was a beginning gastrula with an abnormal number of cells in the blastocœle.

No. 259. Vertical. 1 : 1. Inseminated $3^h\ 22^m$.
N in 8-cell stage $4^h\ 50^m$ (fig. 56), one cell having moved out of position. This cell was smaller than the others; 4 micromeres were formed, one by the smallest cell, between $5^h\ 00^m$ and $5^h\ 02^m$ 16-cell stage with 4 micromeres (3 smaller and one larger).
NN did not become fertilized.

Fig. 56

Fig. 57

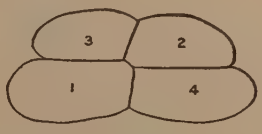

Fig. 58

July 22 No. 260. Vertical. 1 : 4. Inseminated $9^h\ 40^m$.
N regular 2-cell; in 4-cell stage at $12^h\ 01^m$ 2 cells were smaller and 2 larger; into an irregular 8-cell at $12^h\ 02^m$. Lost in transfer.
NN amphiaster appeared in short axis of cell. Regular 2-cell; 4-cell $11^h\ 29^m$, 2 of these cells fused and developed no further; the other 2 divided to 4, a micromere was formed by one cell, and the others divided equally. This was a slightly modified half cleavage.

No. 261. Vertical. 1 : 1. Inseminated $10^h\ 00^m$.
Amphiaster appeared in shorter axis in both fragments. It then rotated.
N 2-cell $11^h\ 06^m$; 4-cell $11^h\ 12^m$; 8-cell $11^h\ 32^m$; the cell sizes were in 4 nearly equal pairs.
NN in 2-cell $11^h\ 06^m$, one cell about half the size of the other; 3-cell $11^h\ 35^m$, the smaller cell alone having divided.
Both were lost in transfer.

No. 262. Vertical. 3 : 1. Inseminated $10^h\ 10^m$.
Amphiaster in shorter axis in both fragments.
N 2-cell $11^h\ 25^m$; 4-cell $11^h\ 42^m$; 8-cell $12^h\ 05^m$; one of the cells

July 22 No. 262 (cont.).
forming a clear protrusion which finally became about half the size of the cell by a regular cell division. 2 micromeres were separated from lower cells and lay adjacent to the clear cell. The fourth lower cell divided equally. 2 of the lower cells therefore formed micromeres and 2 divided equally. The 4 mesomeres divided regularly to 8 giving a 16-cell stage.
NN formed amphiaster, but did not divide.

No. 264. Vertical. 1 : 10. Inseminated 10^h 53^m.
N in 2-cell 11^h 39^m; 4-cell 12^h 02^m; 8-cell 12^h 30^m; 4 micromeres were formed at 12^h 45^m (2 smaller than the other 2); the 4 mesomeres divided to 8, giving a 16-cell stage.
NN in 2-cell 12^h 02^m; regular 4- and 8-cell; formed 2 large and 2 small micromeres 1^h 10^m.

No. 265. Vertical, very slightly oblique. 1 : 17. Inseminated 11^h 18^m.
N rounded up and formed slightly separated membrane; no development 12^h 05^m; in 2-cell 12^h 35^m; irregular 4-cell; 6-cells 1^h 45^m; 7-cell 1^h 50^m; 8-cell 2^h 20^m; formed 4 micromeres at 2^h 45^m.
NN regular 2-cell; 4-cell 12^h 35^m; 8-cell 1^h 05^m; formed 4 micromeres; in 16-cell stage 1^h 17^m.

No. 266. Vertical. 7 : 1. Inseminated 1^h 47^m.
N 2-cell, amphiasters for second division, 2^h 38^m; 4-cell 2^h 47^m; 8-cell 2^h 59^m, 4 cells smaller than the other 4; 3 micromeres formed, 4 mesomeres divided to 8, and one lower cell divided equally at 3^h 18^m (fig. 12).
NN 2-cell 2^h 38^m; 4-cell 2^h 58^m; 8-cell 3^h 25^m; each cell divided equally giving 16 cells at 3^h 40^m. July 23, 8^h 00^m a.m., normal gastrula; very small skeletal spicules.

No. 267. Vertical. 1 : 1. Inseminated 2^h 34^m.
N regular 2-cell; 4-cell 3^h 10^m; in 3 cells 3^h 25^m; 9 cells 3^h 50^m; all divided equally 3^h 55^m.
July 23, 8^h 00^m a.m., active blastula with one mesenchyme cell in the blastocœle (fig. 57).
NN formed a membrane but did not divide.

No. 268. Vertical. Cut around the nucleus. 1 : 2. Inseminated 2^h 34^m.
N 2-cell 3^h 10^m; 4-cell 3^h 42^m; into 8-cells 4^h 00^m, 4 small cells lying above 4 larger. Cell 1 (fig. 58) divided equally at 4^h 25^m; cell 2 at 4^h 26^m; cells 3 and 4 at 4^h 27^m; the 4 cells behind these also divided equally giving a 16-cell stage without micromeres.
NN irregular 2-cell 3^h 30^m; 3-cell 4^h 00^m; 5-cell (1 of the first 2 blastomeres did not continue to divide); 8 cells against one large blastomere at 4^h 39^m. These represented half of a 16-cell stage without micromeres. July 23, disintegrated.

No. 269. Horizontal. 18 : 1. Non-nucleated fragment included the animal polar region. Inseminated 2^h 55^m.
N 2-cell 3^h 43^m, 4-cell 4^h 15^m; 8-cell 4^h 37^m, consisting of 4 larger and 4 smaller cells. The 4 larger divided equally at 4^h 41^m; the 4 lower each formed a micromere at 4^h 42^m. July 23, 8^h 00^m a.m. normal gastrula.
NN amphiaster 3^h 43^m; 2-cell 4^h 00^m; 4-cell 4^h 15^m; 8-cell 4^h 40^m; one cell having a slight protrusion. 4 micromeres were formed at 4^h 55^m, the 4 mesomeres dividing to 8 simultaneously with the formation of micromeres, giving a 16-cell stage. July 23, 8^h 00^m a.m. disintegrated.

August 5 No. 271. Diagonal. 9 : 1 Non-nucleated fragment included the animal pole. Inseminated 10^h 38^m.

N in 2-cell 11^h 22^m; 4-cell 11^h 50^m; 8-cell 12^h 05^m; 4 of the cells smaller than the other 4; the 4 smaller formed micromeres, 2 having twice the diameter of the other 2, at 12^h 20^m; the 4 larger cells divided equally at 12^h 20^m. Polarity was shifted through about 40° (fig. 33).

NN not divided 11^h 50^m; not divided 12^h 07^m.

No. 272. Diagonal. 50 : 1. The non-nucleated fragment included the region surrounding the animal pole. Inseminated 10^h 45^m.

N regular 2-cell; 4-cell 11^h 25^m, 2 of the 4 fused 11^h 35^m; in 8 cells 11^h 43^m; gone back to 6-cell 11^h 57^m; 1 of the 6 formed a micromere and then divided equally, 11^h 59^m; another cell divided unequally and the sixth equally at 12^h 00^m, giving a 12-cell stage with 2 micromeres of unequal size.

NN protrusion formed; irregular cleavage.

No. 273. Diagonal. 9 : 1. Non-nucleated fragment includes the animal polar region. Inseminated 11^h 04^m.

N regular 2-cell; 4-cell 11^h 49^m; 8-cell 12^h 10^m; formed 2 micromeres, giving a 10-cell stage.

NN did not form a membrane; not divided at 11^h 49^m nor at 12^h 10^m.

No. 274. Diagonal. 9 : 1. Non-nucleated fragment includes the animal polar region. Inseminated 11^h 15^m.

N regular 2- and 4-cell; in 8-cell 1^h 00^m; formed one small and 3 large micromeres; followed to 20-cell stage.

NN did not develop.

No. 275. Diagonal. 20 : 1. Non-nucleated fragment included the animal polar region. Inseminated 4^h 00^m.

N 2-cell 4^h 52^m; 4-cell 5^h 10^m, 2 cells slightly smaller than others; these gave an 8-cell stage in which there was a group of 4 cells of equal size, and 2 pairs, one of larger and one of smaller cells. The 4 cells of equal size each gave rise to a micromere at 5^h 52^m; the other 4 cells divided equally at the same time, giving 2 pairs of mesomeres smaller than the other 2 pairs.

August 8, 8^h 00^m a.m. normal gastrula with mesenchyme, skeletal spicules and pigment.

NN regular 2- and 4-cell; in 8-cell 6^h 10^m; all divided equally between 6^h 25^m and 6^h 27^m.

August 6, 8^h 00^m a.m. blastula with 4 mesenchyme cells.

No. 276. Diagonal. 10 : 1. Non-nucleated fragment included the animal polar region. Inseminated 4^h 39^m.

N simultaneous 4-cell 5^h 16^m; 8-cell 5^h 45^m; divided to 12-cell having 8 cells lying between an upper 4 and a lower 4, August 6, 8^h 00^m a.m. evaginate gastrula.

NN not divided 5^h 16^m; 4-cell 5^h 45^m; August 6, 8^h 00^m a.m., blastula with mesenchyme.

No. 277. Diagonal. 6 : 1. Nucleus in vegetal fragment. Inseminated 4^h 55^m.

N 4 micromeres (2 large, 2 small), 4 macromeres and 4 mesomeres, 6^h 25^m; 20-cell 6^h 32^m. August 6, 8^h 00^m a.m., gastrula with shallow archenteron; more than the normal amount of mesenchyme; no skeletal spicules.

NN 5 micromeres, 4 macromeres, 4 mesomeres, 6^h 33^m; the fifth micromere was formed by one of the macromeres after the usual 4 had been formed. August 6, 8^h 00^m a.m., normal gastrula with skeletal spicules.

August 6 No. 278. Diagonal. 1 : 5. Nucleus in animal fragment. Inseminated $10^h\ 35^m$.

N 2-cell $11^h\ 11^m$; 4-cell $11^h\ 45^m$; 8-cell $11^h\ 49^m$ (cells of equal size). All cells divided equally at $12^h\ 05^m$ giving 16 cells. August 7, $9^h\ 00^m$, blastula with 6 mesenchyme cells (fig. 44).

NN into 2-cell $11^h\ 18^m$; 4-cell $11^h\ 45^m$ (2 larger and 2 smaller cells); into 7 cells $11^h\ 50^m$, one of the blastomeres of the 4-cell not dividing; this blastomere formed a small cell (micromere?) at $12^h\ 00^m$; 3 undoubted micromeres were formed at $12^h\ 10^m$, the 3 mesomeres dividing equally at the same time. No further divisions at $12^h\ 25^m$. August 7, $9^h\ 00^m$ a.m., gastrula with one well-developed skeletal rod, and a spicule only on the opposite side (fig. 45). Gastrocœle rather crowded with mesenchyme cells.

No. 279. Diagonal. 10 : 1. Non-nucleated fragment contained the animal polar region. Inseminated $10^h\ 53^m$.

N 2-cell $11^h\ 35^m$; 4-cell $11^h\ 58^m$; 8-cell $12^h\ 19^m$ (fig. 59). Three cells each formed a micromere $12^h\ 42^m$; all others divided equally $12^h\ 55^m$ to $12^h\ 56^m$, giving 16-cell stage.

Fig. 59

August 7, $9^h\ 00^m$ a.m. very good late gastrula or beginning pluteus (fig. 46); 2 large triradiate spicules. Pigment-containing cells among the clear mesenchyme cells and in the ectoderm.

NN into 2-cell $11^h\ 47^m$; in 4-cell $12^h\ 15^m$; into 8-cell $12^h\ 30^m$. The cleavage pattern is exactly like that of N; its succeeding divisions were the same; 3 micromeres and 13 other cells.

August 7, $9^h\ 00^m$ a.m., blastula with mesenchyme; no skeletal spicules (fig. 47).

No. 280. Diagonal. 5 : 1. The non-nucleated fragment contained the vegetal polar region. Inseminated $3^h\ 30^m$.

N early amphiaster $4^h\ 05^m$; 2-cell $4^h\ 30^m$; 4-cell $4^h\ 39^m$; 8-cell $4^h\ 59^m$, 4 larger cells and 2 pairs of smaller cells (fig. 36). Each of the smaller cells formed a micromere and the larger cells divided equally at $5^h\ 22^m$. Each macromere was of about the same size as each of the 8 mesomeres.

NN did not form a membrane; had not divided at $4^h\ 30^m$.

No. 281. Diagonal. 5 : 1. The non-nucleated fragment contained the vegetal polar region. Inseminated $3^h\ 50^m$.

N no membrane; no development.

NN amphiaster $4^h\ 30^m$; 2-cell $4^h\ 57^m$; 4-cell $5^h\ 05^m$; 8-cell $5^h\ 30^m$, 4 cells being smaller than the other 4. The 4 large cells divided at $5^h\ 45^m$, one equally, while 3 formed micromeres. The 4 mesomeres divided to 8, only slightly larger than the micromeres, at $5^h\ 47^m$.

No. 282. Diagonal. 1 : 1.1. Nucleus near vegetal pole. Inseminated $3^h\ 55^m$.

N 2-cell $4^h\ 31^m$; 4-cell $4^h\ 59^m$; 8-cell, 4 cells smaller than the other 4. The 4 smaller cells divided unequally; the larger 4 equally. 16-cell, 4 micromeres.

NN irregular 2-cell; irregular 4-cell $4^h\ 59^m$; cells well rounded $5^h\ 05^m$; 7 cells, 1 of which was large and contained 2 amphiasters. Each of 6 cells divided equally at $5^h\ 32^m$. The large cell divided between the amphiasters at $5^h\ 34^m$; then each of the resulting cells divided at $5^h\ 36^m$. 16-cell, no micromeres.

No. 283. Diagonal. 5 : 1. Nucleus in animal fragment. Inseminated $4^h\ 01^m$.

N amphiaster $4^h\ 37^m$; 4-cell $5^h\ 06^m$; 8-cell, 4 cells smaller than the other 4; 2 of smaller cells formed micromeres, 2 divided unequally at $5^h\ 58^m$. The 4 larger cells divided equally at $5^h\ 59^m$,

Aug. 6 No. 283 (cont.).
giving a 16-cell stage with 2 large and 2 small micromeres. August 7, 9^h 00^m a.m., gastrula, with mesenchyme cells and skeletal spicules. Wall on one side thickened (fig. 48).
NN 2-cell 5^h 06^m. Cleavage not followed further. August 7, 9^h 00^m a.m., slowly moving gastrula with compact, apparently solid archenteron; mesenchyme cells packed around the archenteron; 2 very small triradiate spicules (fig. 49).

No. 284. Diagonal. 4.4 : 1. The non-nucleated fragment included the vegetal polar material. Inseminated 4^h 10^m.
N 2 micromeres, 2 macromeres, 2 lower cells that divided equally, 8 mesomeres, giving a 16-cell stage with 2 micromeres. August 7, 9^h 00^m a.m., fairly normal gastrula; too much mesenchyme.
NN did not form membrane; no cleavage.

No. 285. Diagonal. 1 : 4. Nucleus in vegetal fragment. Inseminated 4^h 25^m.
N 3 small micromeres, 1 lower cell that divided equally, and 8 mesomeres; a 16-cell stage with 3 micromeres. August 7, 9^h 00^m a.m. partially disintegrated gastrula.
NN 8-cell stage consisted of 6 large and 2 small blastomeres. The 2 small cells formed micromeres; the remaining cells divided equally, giving a 16-cell stage with 2 micromeres. August 7, 9^h 00^m a.m., partially disintegrated gastrula.

EARLY CLEAVAGE OF LYTECHINUS EGGS

LYTECHINUS EGGS ISOLATED AND INSEMINATED IN 1 C.C. OF SEA-WATER

July 23, 1926

Female A:

(1) Inseminated 11^h 06^m. Poor membrane. In 2-cell 12^h 00^m; 4-cell 12^h 50^m; 16-cell 1^h 20^m (2 micromeres from 2 vegetative blastomeres, 2 blastomeres divided equally, 8 mesomeres).
(2) Inseminated 11^h 06^m. Good membrane. Into 4-cell 12^h 02^m. 4 micromeres, 4 macromeres, 8 mesomeres 12^h 53^m.
(3) Inseminated 11^h 06^m. Good membrane. In 2-cell 12^h 02^m; 16-cell, no micromeres, 1^h 03^m.
(4) Inseminated 11^h 06^m. Fair membrane. In 4-cell 12^h 39^m. 4 micromeres forming from lower cells and 4 upper cells dividing to 8 mesomeres at 1^h 08^m.
(5) Inseminated 11^h 06^m. Fair membrane. 2 micromeres, 2 vegetative blastomeres divided equally, 4 mesomeres at 1^h 10^m.

Female B:

(6) Inseminated 11^h 15^m. No membrane. No development.
(7) Inseminated 11^h 15^m. No membrane. No development.
(8) Inseminated 11^h 15^m. No membrane. No development.

Female C:

(9) Inseminated 11^h 22^m. No membrane. No development.
(10) Inseminated 11^h 22^m. Good membrane. 4-cell 12^h 45^m, 4 micromeres, one slightly delayed in formation, 4 macromeres and 8 mesomeres at 1^h 33^m. The fourth micromere formed was smaller than the others.
(11) Inseminated 11^h 22^m. Good membrane. 4-cell 12^h 45^m; 4 micromeres, 4 macromeres and 8 mesomeres 1^h 25^m.
(12) Inseminated 11^h 22^m. Good membrane. 4-cell into 8, 12^h 46^m; too advanced to follow 1^h 35^m.

LYTECHINUS EGGS ISOLATED AND INSEMINATED IN 1 C. C. OF SEA-WATER

July 24, 1926

Female A:

(1) Inseminated 10^h 29^m. No membrane. No development. No evidence of entrance of spermatozoön.

(2) Inseminated 10^h 29^m. Excellent membrane. 2-cell 11^h 20^m; 4-cell 11^h 31^m; 8-cell 11^h 50^m, 4 of the cells being distinctly smaller than the other 4; 2 large and 2 small cells forming micromeres at 12^h 08^m; mesomeres dividing 12^h 09^m (4 micromeres).

(3) Inseminated 10^h 29^m. Excellent membrane. 2-cell 11^h 20^m; 8-cell 11^h 12^m, 4 of the cells slightly smaller than the other 4. Failed in development. Cytolized.

(4) Inseminated 10^h 43^m. Excellent membrane. Fine amphiaster 11^h 22^m; in 8-cell 12^h 22^m; all cells divided equally at 12^h 41^m except one which formed a micromere.

(5) Inseminated 10^h 43^m. Excellent membrane. Egg elongating slightly in direction of long axis of spindle. Division beginning 11^h 24^m; division completed 11^h 25^m = 42 minutes from insemination; 8 cells 11^h 22^m, 2 micromeres at 12^h 00^m; 2 lower cells divided equally 12^h 50^m; 4 upper cells divided equally to 8 mesomeres.

(6) Inseminated 10^h 43^m. Excellent membrane. 2-cell 11^h 27^m; 4 micromeres formed. Later development, 3 of the micromeres each formed a smaller one, 1 remaining undivided; the 3 which had produced the generation of smaller ones divided equally at 1^h 10^m; the 4th did not divide. There thus resulted a circlet of 7 cells enclosing 3.

Note: The usual division of micromeres in *Lytechinus* is the formation of 4 smaller ones centrally placed, by the unequal division of the original 4, then the equal division of the outer ones, forming a circlet of 8 surrounding 4, 12 in all.

Female B:

(7) Inseminated 10^h 53^m. Fair membrane. Divided by tetraster to 4 cells of unequal size. 11^h 41^m. Very irregular sphere 1^h 15^m.

(8) Inseminated 10^h 53^m. Excellent membrane. 2-cell 11^h 37^m; circlet of 8 micromeres with 4 small ones in center at 1^h 20^m.

(9) Inseminated 10^h 53^m. Poor membrane. No development 11^h 40^m; discarded 1^h 22^m.

(10) Inseminated 11^h 08^m. Excellent membrane. Egg elongating and division beginning 11^h 48^m; regular cleavage; 4 micromeres forming the second generation of micromeres 1^h 25^m.

(11) Inseminated 11^h 08^m. Excellent membrane. 4 micromeres; 20-cell stage, normal, 1^h 35^m.

(12) Inseminated 11^h 08^m. Excellent membrane. Dividing to 2-cell 11^h 45^m (37 minutes after insemination). 4 micromeres formed; in the further development of these all gave off a smaller micromere, then 3 divided equally, 1 remaining undivided; there was then a circlet of 7, one of which was larger than the other 6, enclosing a group of 4. Micromeres sinking into the wall 1^h 48^m.

LYTECHINUS EGGS ISOLATED AND INSEMINATED IN A SYRACUSE WATCH-GLASS CONTAINING 15 C.C. OF SEA-WATER

July 25, 1926, a.m.

Female A:

(1) Inseminated 10^h 10^m. Ragged membrane. 2-cell 10^h 55^m; 4-cell 11^h 20^m, 2 cells being slightly smaller than other 2. Into 8-cell 11^h 29^m; 4 micromeres at 11^h 50^m, the mesomeres dividing nearly simultaneously, giving a total of 16 cells.

Female A (cont.):

(2) Inseminated 10^h 10^m. Excellent membrane. Amphiaster 10^h 56^m; in 2-cell 11^h 22^m; dividing to 4 at 11^h 22^m; lost when transferring in late 8-cell at 12^h 05^m.

(3) Inseminated 10^h 10^m. Excellent membrane. No development.

Female B:

(4) Inseminated 10^h 31^m. Excellent membrane. 4 micromeres, 4 macromeres, 8 mesomeres.

(5) Inseminated 10^h 31^m. Excellent membrane. 4 micromeres, 4 macromeres, 8 mesomeres.

(6) Inseminated 10^h 31^m. Excellent membrane. 8-cell 12^h 23^m; 4 micromeres 12^h 28^m; 4 upper cells divided to 8 mesomeres simultaneously with formation of micromeres.

Female C:

(7) Inseminated 10^h 51^m. Excellent membrane. No development.

(8) Inseminated 10^h 51^m. Excellent membrane. No development.

(9) Inseminated 10^h 51^m. Excellent membrane. No development.

Female A (Same as used for 1 to 3):

(10) Inseminated 11^h 05^m. Excellent membrane. 3 micromeres, one from each of 3 megameres; 1 lower cell divided equally; 4 upper cells divided to 8 mesomeres 1^h 50^m.

(11) Inseminated 11^h 05^m. Excellent membrane. 4 micromeres 12^h 40^m, 4 lower cells, 4 upper cells dividing to 8 mesomeres at 12^h 45^m.

July 25, p.m.

Room temperature 34° C.

Temperature of sea-water when brought in 29° C.

Temperature of 100 c.c. sea-water after standing on table one hour 30.5° C.

Female A (new female):

(1) Inseminated 1^h 54^m. Poor membrane. Going to 3-cell 2^h 35^m; irregular 5-cell at 3^h 12^m; 7-cell 3^h 30^m (4 small and 3 large cells); 8-cell 3^h 31^m; 10-cell 3^h 40^m.

(2) Inseminated 1^h 54^m. Fair membrane. Amphiaster 2^h 38^m; 2-cell 2^h 41^m; 4-cell 3^h 17^m; 3 micromeres from 3 macromeres; 1 lower cell divided equally; 8 mesomeres.

(3) Inseminated 1^h 54^m. Excellent membrane. 2-cell 2^h 39^m; 4-cell 3^h 03^m; 8-cell 3^h 27^m; 3 micromeres from 3 lower cells; 1 lower cell divided equally; 8 mesomeres 4^h 30^m. Temperature of water in Syracuse watch-glass containing this egg 32° C. at 4^h 30^m.

(4) Inseminated 2^h 06^m. No membrane. No development.

(5) Inseminated 2^h 06^m. Good membrane. 4 micromeres, 8 macromeres, 8 mesomeres.

(6) Inseminated 2^h 06^m. Good membrane. 4 micromeres, 8 macromeres, 8 mesomeres; 4 small micromeres from first set at 4^h 22^m.

July 26

Temperature of sea-water in large jar 29.5° C.

Air temperature on table 33° C.

Temperature of 1 c.c. sea-water in Bureau of Plant Industry watch-glasses after standing covered on table 2.5 hours 31.2° C.

Temperature of 15 c.c. sea-water in Syracuse watch-glass after standing covered on table 2.5 hours 32° C.

July 28

Mass insemination of *Lytechinus* eggs in 500 c.c. sea-water at $11^h\ 25^m$. Beginning to go into 2 cells $12^h\ 00^m$. In 8 cells $12^h\ 55^m$.

(1) 1 lower cell divided equally . . . . . 2 cells
3 lower cells each formed a micromere 6
8 mesomeres 8

Total . . . . . 16 $1^h\ 07^m$

(2) 4 micromeres, 4 macromeres, 4 mesomeres . . . . . $1^h\ 12^m$
(3) 8 cells that divided equally to 16 . . . . . $1^h\ 18^m$
(4) 2 small micromeres, 2 larger (1.5 times the diameter of the smaller), 4 macromeres, 8 mesomeres . . . . . $1^h\ 22^m$
(5) 4 micromeres, 4 macromeres, 4 mesomeres dividing to 8 . . . . . $1^h\ 26^m$
(6) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $1^h\ 26^m$
(7) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $1^h\ 27^m$
(8) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $1^h\ 28^m$
(9) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $1^h\ 29^m$
(10) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $1^h\ 34^m$
(11) 4 micromeres of first generation, 4 micromeres of second generation, 8 macromeres, 16 mesomeres . . . . . $1^h\ 42^m$
(12) Same as 11 . . . . . $1^h\ 44^m$
(13) 4 micromeres, 8 macromeres, 8 mesomeres . . . . . $1^h\ 46^m$
(14) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $1^h\ 47^m$
(15) 4 micromeres, 8 macromeres forming, 8 mesomeres . . . . . $1^h\ 50^m$

July 30

Mass insemination of *Lytechinus* eggs in 500 c.c. sea-water $3^h\ 45^m$. Excellent membranes and an unusually thick layer of ectoplasm. Temperature of water on table 30° C. Some eggs in first division at $4^h\ 24^m$, *i. e.* 39 minutes after insemination.

(1) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 20^m$
(2) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 25^m$
(3) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 26^m$
(4) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 27^m$
(5) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 29^m$
(6) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 30^m$
(7) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 31^m$
(8) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 32^m$
(9) 2 micromeres, 6 macromeres, 8 mesomeres . . . . . $5^h\ 33^m$
(10) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 37^m$
(11) 4 micromeres (3 very large), 4 macromeres, 8 mesomeres . . . . . $5^h\ 39^m$
(12) 4 micromeres, 4 macromeres, 8 mesomeres . . . . . $5^h\ 40^m$

Development of Arbacia egg fragments of second period

Case and plane of cut	Position of nucleus	Character of cleavage					
		Nucleated fragment	No. of cells	No. of micromeres	Non-nucleated fragment	No. of cells	No. of micromeres
Sept. 3:							
No. 1H	A1	Too compact to follow	8	?	One posterior cell divided equally	12	3
2H	A0	Cleavage irregular			Cleavage irregular		
3H ⊥	V1		12	4	Undetermined		
4H ⊥	A1		16	None		16	4
5H ⊥	A1½	Cleavage irregular				16	2
Sept. 4:							
1H ⊥	A ½	2 rows of 8 cells each	16	None	Endoplasmic bud		
2H ⊥	V1	Endoplasmic bud			Cleavage irregular		
3D	V0	Endoplasmic bud			Endoplasmic bud		
4H ⊥	A2	Endoplasmic bud			Endoplasmic bud		
5H ⊥	A2	Excellent cleavage	16	None	Excellent cleavage	16	None
6H ⊥	A1½	Tetraster	4		No cleavage		
7H ⊥	A1	Endoplasmic bud			Endoplasmic bud		
Sept. 6:							
1H	V2	Cleavage irregular	4		Cleavage irregular	4	
2H ⊥	A1	Polyspermy; no cleavage			Polyspermy; no cleavage		
3H ⊥	V1	Lost			No cleavage		
4H ⊥	A1½	Polyspermy; cleavage irregular	6		Polyspermy; cleavage irregular	4	
5H ⊥	V1½	Cleavage irregular			Cleavage irregular		
6H ⊥	V1	Cleavage regular	12	4	One cell of 8 cell stage formed a micromere	9	1
7H ⊥	A ¼	Cleavage as in 6NN	9	1	Cleavage irregular		
Sept. 7:							
1 ⊥	A1	Endoplasmic bud			Endoplasmic bud		
2 ⊥	A ¼	No further cleavage	3		Irregular cleavage	3	
3 ⊥	A1	Endoplasmic bud	2		Endoplasmic bud	2	
4 ⊥	A1½	Endoplasmic bud	2		Endoplasmic bud	2	

Development of Arbacia egg fragments of second period—(Cont.)

Case and plane of cut	Position of nucleus	Character of cleavage					
		Nucleated fragment	No. of cells	No. of micromeres	Non-nucleated fragment	No. of cells	No. of micromeres
Sept. 8:							
1H ⊥	V1	2 cells protruding through membrane	12	0	Cells unequal in size	16	2
2H ⊥	V1½	Polyspermy; cleavage irregular	6	.....	Polyspermy; cleavage irregular	2	.....
3H ⊥	A1½	Tempo of cleavage irregular	16	4	Cleavage irregular	4	.....
4H ⊥	V2½	Cleavage irregular	.....	.....	Good cleavage till 8 cells; disintegrated	8	.....
5H ⊥	A2½	2 cells of 8 cell slightly smaller	16	0	No development	.....	.....
6H ⊥	V1	Endoplasmic bud	.....	.....	Cleavage irregular	.....	.....
7H	A0	Cleavage irregular	.....	.....	Cleavage irregular	.....	.....
8H ⊥	A2½	Cleavage irregular	.....	.....	Cleavage irregular	.....	.....
Sept. 9:							
1H ⊥	V1½	Endoplasmic bud	16	2	Endoplasmic bud	16	0
2H ⊥	A1	Cleavage abnormal	?	?	Endoplasmic bud	16	4
3H ⊥	A1½	Endoplasmic bud	.....	.....	Endoplasmic bud	.....	.....
4H	A3	Cleavage fair	12	3	Cleavage irregular	.....	.....
5H ⊥	A2	Endoplasmic bud	.....	.....	Endoplasmic bud	.....	.....
6H ⊥	A1½	Endoplasmic bud	.....	.....	Endoplasmic bud	.....	.....
7H ⊥	A ½	Endoplasmic bud	.....	.....	Endoplasmic bud	.....	.....
Sept. 10:							
1H ⊥	V1½	Was not inseminated	.....	.....	Endoplasmic bud	16	4
2H ⊥	V ½	Endoplasmic bud	12	0	Endoplasmic bud	16	0
3H ⊥	A2	Endoplasmic bud in 8 cell stage	12	4	Endoplasmic bud	12	4
4H ⊥	A2	Endoplasmic bud	8	.....	No division after 8 cell stage	8	.....
5H ⊥	A2	No division after 8 cell stage	8	.....	No division after 8 cell stage	8	.....
6H	A2	Endoplasmic bud	.....	.....	Endoplasmic bud	.....	.....
7H ⊥	V3	Endoplasmic bud	.....	.....	Endoplasmic bud	.....	.....

ABBREVIATIONS

H, Horizontal.

⊥, Perpendicular to line connecting nucleus with center of egg.

A, Animal fragment.

V, Vegetal fragment.

0, ¼, ½, 1, 1½, 2, 2½, 3; distance of nucleus from plane of section reckoned in nuclear diameters. AO indicates that the nucleus was in the animal fragment against the plane of section. A¼ indicates that the nucleus was in the animal fragment and was ¼ of its diameter from the plane of section; V1½ indicates that the nucleus was in the vegetal fragment and was 1½ times its diameter from the plane of section, etc.

II

ACTIVATION OF THE EGGS OF ECHINOMETRA MATHÆI BY SPERMS OF THE CRINOIDS COMATULA PECTINATA AND COMATULA PURPUREA

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Five text-figures

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ACTIVATION OF THE EGGS OF ECHINOMETRA MATHÆI BY SPERMS OF THE CRINOIDS COMATULA PECTINATA AND COMATULA PURPUREA

NATURE OF THE MATERIAL

The classical research in this field is that of Godlewski ('06), who succeeded in fertilizing the eggs of three species of Echinoids with the sperms of *Antedon rosacea.* The results of the activation of eggs of *Echinometra mathœi* by the spermatozoa of the crinoids *Comatula pectinata* and *Comatula purpurea,* described in this paper, were obtained in September and October, 1913, at Mer, in the Murray Islands, Torres Strait. Although a satisfactory study of the early development of *Echinometra mathœi* was made at this time, it is a matter of regret that no material for either the study of the development of the crinoids used nor for the reciprocal Echinoid ♂ × Crinoid ♀ cross could be obtained.

In both crosses made there was a lack of uniformity in extent of development. Many eggs developed normally up to an early gastrula and could not be distinguished from pure *Echinometra* embryos of the same stage of development. The majority of the cross activated eggs, fully 85 per cent, became aberrant in their development at various points between late cleavage and early gastrula. Even in the most normal embryos, developmental block became evident during the early gastrula stage. This block resulted in complete inhibition of normal processes of development, although the larvæ retained a high degree of viability.

TECHNIQUE

The technique of the experimental work, although simple, insured the dependability of the results. The precautions taken against chance fertilization gave maximum protection. On September 26 an *Echinometra* was obtained which began to spawn as soon as it was brought into the laboratory. This sea-urchin was at once washed for one minute in fresh water, then rinsed in sterilized sea-water and placed, apical surface down, in a dry finger bowl, where it continued to discharge its eggs.

All glassware used had been boiled and air dried. The sea-water used had been heated to 70° C., and allowed to cool to room temperature. All instruments and pipettes, after being used once, were dropped into boiling water before being used again.

From time to time the eggs, which continued to be discharged through the genital pores of the spawning female, were transferred to finger-bowls of

sterilized sea-water. The water in these bowls was changed twice during the first half hour. Each separate portion of the eggs obtained in this manner constituted a "stock" from which eggs were transferred to other bowls of sea-water in such quantity that only a thin layer covered the bottom of each bowl. One of the dishes of each lot was kept as a control. No formation of membranes and no segmentation occurred in any of the controls.

The sperms were obtained by cutting 7 to 10 pinnules from an arm of a male crinoid into a watch-glass containing 5 c.c. of sterilized sea-water, after the crinoid had been rinsed in fresh water and then in sterilized sea-water. As soon as the white mass of sperms had exuded from the cut ends of the pinnules, the pinnules were removed from the watch-glass.

It was possible to obtain activation and development of a small number of eggs, less than one per cent, by inseminating the eggs within half an hour after their extrusion from the ovaries. By allowing the eggs to stand in sea-water the percentage of activable eggs increased during the first 2 1/2 hours, when it reached a maximum of 50 to 60 per cent. Between 2 1/2 and 4 hours the number of activable eggs remained fairly constant; after 4 hours there was a rapid decline.

About 2 c.c. of diluted sperm suspension was added to the bowl of prepared eggs in each attempted activation and the mixture stirred gently. After half an hour the supernatant water was poured from the inseminated eggs and replaced by sterilized sea-water. This process was repeated several times, so that the eggs were freed, as completely as possible, from the influence of dead spermatozoa.

After 10 hours the swimming blastulæ were poured into another finger bowl in order to avoid the injury of the cultures by the disintegrating unfertilized eggs.

The identification of the species used in the work was made by Dr. H. L. Clark.

OBSERVATIONS ON EARLY DEVELOPMENT OF CROSS-ACTIVATED EGGS

Activation, with its resulting membrane formation, never exceeded 60 per cent of the inseminated eggs. Activation was never simultaneous in all the eggs. In some the activation was immediate, in others delayed, so that there was a gradual increase in number of eggs with membranes during the first fifteen minutes following insemination. As a result of this irregular activation there was considerable irregularity in the time of the appearance of the first cleavage furrow, this visible evidence of beginning cleavage appearing in from one hour and five minutes, as in the species fertilized egg, to approximately one hour and twenty minutes after insemination.

The tempus of cleavage and of early development was as in the species fertilized eggs. In a small percentage of the eggs, cleavage was irregular

from the beginning. The normal blastulæ and blastulæ with mesenchyme were not distinguishable from those obtained from species fertilized *Echinometra* eggs. From the stage of blastulæ with mesenchyme onward, the abnormality of the cultures increased until, at 20 hours, there were but few gastrulæ of the normal *Echinometra* type. In these exceptional gastrulæ the characteristic tri-radiate skeletal spicules appeared in their proper position at the sides, adjacent to the base of the archenteron, as in normal *Echinometra* gastrulæ (fig. 1). Normal development, of the *Echinometra* type, did not continue beyond this stage, the pyramidal form, characteristic of the young pluteus, never being attained.

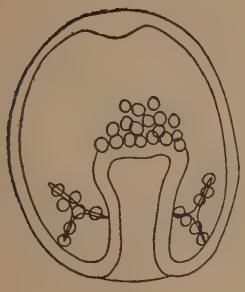

Fig. 1

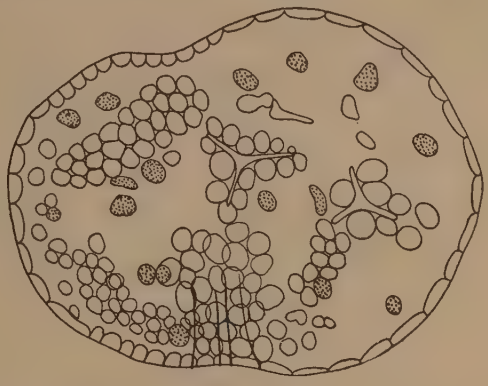

Fig. 2

ABERRANT DEVELOPMENT OF THE HYBRID LARVÆ

Comatula pectinata ♂ × Echinometra mathæi ♀

One series of activations was made with spermatozoa from *Comatula pectinata*. The first general departure from the normal course of development was coincident with the process of formation of mesenchyme. The blastocœle became filled with cells, among which were scattered a few abnormally large cells containing granules of red pigment. Many of these larvæ remained alive into the third day without further change of form. No skeletal structures were formed in larvæ of this kind. Ability to develop had been inhibited.

Other larvæ from the same fertilization passed through the process of mesenchyme formation normally and began their process of gastrulation in 20 to 24 hours from the time of activation of the eggs. In these larvæ, as in the normal, skeletal spicules appeared slightly before gastrulation began. The onset of gastrulation, however, seemed to mark another critical period in their lives, for few continued the process normally. The most frequent development, if that word may be applied, consisted in the proliferation of considerable masses of cells from the end of the archenteron. This was probably a modification of the process of formation of secondary mesen-

chyme which occurs in the species fertilized *Echinometra* eggs at 20 hours. The result of this process was essentially an elimination of the archenteron, only a rudiment of this structure, with a narrow and shallow lumen, remaining (fig. 2).

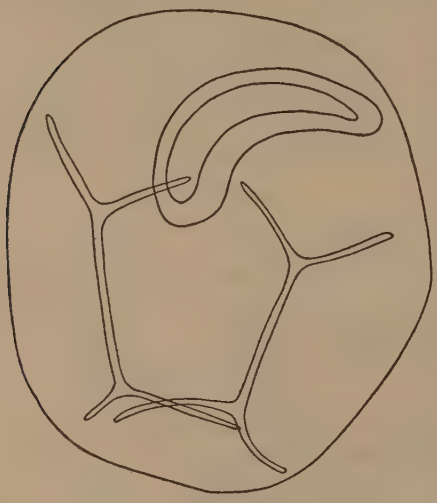

Fig. 3

In other aberrant larvæ of the same age, 67 hours (fig. 3), there was no indication of a blastopore, but a compact mass of cells occupied a median position between the two body rods of a fairly typical skeleton of a young pluteus. A well-developed superficial groove was present in the larva drawn, extending from one side of the larva, posteriorly toward the median line. A groove of this kind was seen in this one larva only.

The general resemblance of these structures to those of certain stages in the development of the larva of *Antedon rosacea* (Seeliger, '93) is obvious, yet it would be unwise to attempt to homologize the structures appearing in these abnormal larvæ with the enterocœlomic vesicle and the vestibule of the larva of *Antedon rosacea*. Furthermore, as we have no knowledge of the embryology of *Comatula pectinata* we have no means of knowing how closely it resembles *Antedon rosacea* in its processes of development.

The illustrations represent the structure of the most active and the oldest larvæ obtained from this cross.

Comatula purpurea ♂ × Echinometra mathæi ♀

Another series of inseminations was made with spermatozoa from *purpurea*. About 50 per cent of the eggs were activated. Cleavage was regular in less than half of the activated eggs, the remainder dividing very irregularly. A small number of the activated eggs reached the blastula stage, but none of these larvæ formed their mesenchyme normally. There was active proliferation of cells from the posterior pole of the blastula and rapid division of these cells within the blastocœle. In the younger stage (fig. 4), the mesenchyme cells were large, and there were a few amœboid cells containing rather coarse granules of red. In a slightly older stage, it was evident from the size of the cells, that most of the increase in number of cells within the blastocœle was due to division of the originally proliferated cells.

About this time the amœboid excretory (?) cells began to die, as was indicated by the disintegration of their granules and the diffusion of their pigment throughout the cell body (fig. 5). This process marked the beginning of high mortality in the culture and the end of all progressive development.

DISCUSSION

Godlewski's ('06) conclusions were based upon a careful and prolonged study of abundant material. The facts that have been presented in the present paper are based on only two fertilizations made on the eggs of one echinoid. Despite this fact it is possible that some comparison of results will be of value.

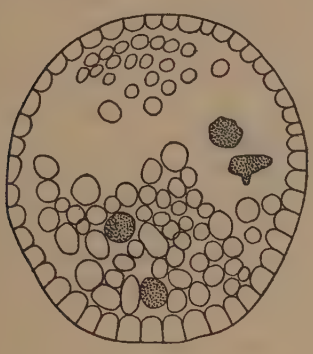

Fig. 4

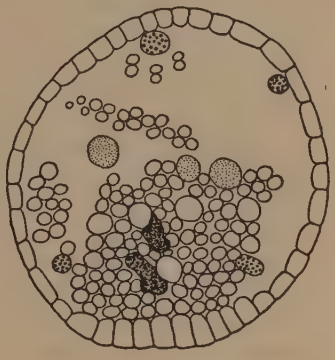

Fig. 5

My observations, so far as they go, confirm those made by Godlewski. They confirm his conclusions as to the tempus of cleavage and as to tendency to remain longer in the blastula stage than purely bred larvæ. The observations are also in accord with those of Godlewski as to critical points in development. Godlewski ('06, p. 612) pointed out that the formation of mesenchymatic tissue, gastrulation, the formation of skeletal anlagen and the further development of the skeleton, are all morphogenetic processes in which the manner of development of the two orders used in the crosses differ.

I obtained no stages of so advanced a stage of development as those obtained by Godlewski from his *Antedon* ♂ × *Echinus* ♀ and *Antedon* ♂ × *Strongylocentrotus* ♀ fertilizations. Success in development of the *Comatula pectinata* ♂ × *Echinometra mathæi* ♀ hybrids approached that of Godlewski's *Antedon* ♂ × *Strongylocentrotus* ♀ cross, surpassed that of his *Antedon* ♂ × *Sphærechinus* ♀ cross, and was distinctly inferior to that of his *Antedon* ♂ × *Echinus* ♀ cross. In other words, many larvæ formed typical *Echinometra* gastrulæ with the characteristic tri-radiate skeletal spicules, but none formed plutei. Even in the most successful examples,

development always became aberrant during the gastrula stage, so that even though a nearly typical skeleton was formed, as in figure 3, in which the body rods, the post-oral rods and the ventral transverse rods of a young pluteus are evident, no post-oral arms pushed out, nor did the larva ever attain the normal pyramidal form of the pluteus.

This generalized comparison is somewhat misleading in that the outcome of a single experiment is compared with the best results of Godlewski's long series of experiments. Godlewski ('06, p. 609) has shown that even in his *Antedon* ♂ × *Echinus* ♀ results, there was great variation in range of development, and that not only does the percentage of eggs that have been inseminated with *Antedon* sperms (and which develop to the stage of the pluteus) vary, but often in such crosses no individuals develop beyond the gastrula. There is no means of knowing whether, with the material which forms the basis of the present paper, the results were exceptionally good or exceptionally poor.

As a matter of fact, in dealing with a cross of this nature, a cross of Crinoid-Echinoid width and, more particularly, a cross in which the egg of an echinoid has been activated by a spermatozoön of a crinoid, one would expect either a larva of the maternal type, or one showing an upset and aberrant development. In the one instance the spermatozoön acts simply as an activating agent. In the other it either produces incomplete activation with a consequent imperfect development, or the fertilization reaction is complete and the nuclear substances of the spermatozoön enter into the series of reactions with the cytoplasm of the egg.

It is obvious that a spermatozoön from a species which has neither primary mesenchyme nor skeletal rods during its first 24 hours of development can carry little in the way of formative stimuli for these structures. What then is to be regarded as evidence of early paternal influence in heterogeneric hybrids of this type?

Loeb ('04) faced this question squarely in his consideration of his Echinoid-Asteroid larvæ and has made an exceedingly satisfactory statement concerning the matter (l. c. p. 53). "Whenever we succeed in the case of hybridization in introducing a positive characteristic of one of the parents into a larva we can be tolerably certain that this is the transmission of a hereditary character, but when we are dealing with a negative characteristic, as in this case, where a skeleton is poorly developed or not developed at all, we can not be equally certain, because a deficiency can be produced by a mere disturbance in the development of the embryo, and can not be considered as a sure indication of the transmission of the hereditary characters of one of the parents."

We may start with the premise that the spermatozoön may activate the egg, or it may activate the egg and transmit to it the determiners of a series of development reactions—the determiners of a course of development.

It is not clear that in a heterogeneric cross of the type under consideration, its influence need be restrictive or inhibitive. Yet, if we consider the facts that have been established by the study of hybrids of this nature, we find that the course of development tends to become aberrant at the stages which mark divergences in type of development between the species crossed.

The echinoid egg, when properly activated by means causing its parthenogenetic development, goes through the more strictly mechanical part of its development normally, and if its segmentation has been normal gives rise to a normal blastula, and in turn to a normal gastrula. But the cross activated egg, even though it segments normally, may become aberrant in its development at the onset of formation of mesenchyme, or in the formation of the skeleton, or in the process of gastrulation, or at the time of transformation of gastrula to pluteus.

Material for a study of reciprocal inter-class crosses would be highly desirable. Reciprocal crosses within the genus, or within the family, are of little value in the study of the influence of the spermatozoön on early development, for the reason that the divergent processes of development are usually not striking enough to afford safe criteria for comparison. Yet it is evident, when we observe the result of some of these intergeneric crosses, that a normally harmonious course of development has been disorganized.

The only crosses that I have found to be of positive value in such a study are those from the interordinal *Lytechinus* ♂ × *Cidaris* ♀, and *Tripneustes* ♂ × *Cidaris* ♀ fertilizations (Tennent '22). These crosses are of positive value for the reason that the spermatozoa are from parents in whose normal development there is an early formation of structures which are formed at a later stage in the development of the egg parent. That is, in *Lytechinus* and *Tripneustes* there is the early formation of mesenchyme and skeleton that is typical of echinoids, while in *Cidaris,* also an echinoid, whose eggs were used, there is the late formation of mesenchyme that is typical of *Antedon.*

In other words, this Echinoid × Echinoid cross has given opportunity for the study of processes that we may expect to find in Echinoid ♂ × Crinoid ♀, and Echinoid ♂ × Asteroid ♀ crosses.

The logical test of the influence of the spermatozoön on early development, to be conclusive, should be a test that will give positive evidence. The demonstration of ability to produce mesenchyme, or lack of ability to produce mesenchyme, by an egg which in its normal development following species fertilization, produces no mesenchyme from the posterior wall of the blastula, is evidence of this nature. Evidence of this type was given by the obvious influence of the spermatozoön on the early development of the *Cidaris* egg following fertilization by *Lytechinus* or *Tripneustes* spermatozoa.

Fischel's ('15) conclusions to the effect that in early development there are two epochs, the first characterized by material separation of the egg mass,

the second characterized by increasingly complex chemical changes, that is, that the early processes of development are largely mechanical, while the later are chemical, find strong support in the evidence from cross activation.

Fischel's generalization corresponds to Roux's earlier conclusion that in development there are two periods, the first a period of primary or directly inherited development, the second a period of secondary or indirectly inherited development under the influence of formative stimuli.

The results of the work here described support my conclusions ('22) that an orderly series of developmental reactions is disorganized by foreign nuclear material at the time when divergence of two systems of development occurs, and that it is through its organization and its capacity as substrate that the course of development of the egg is fixed.

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III

EARLY DEVELOPMENT AND LARVAL FORMS OF THREE ECHINOIDS OF THE TORRES STRAIT REGION

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Fifteen text-figures

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EARLY DEVELOPMENT AND LARVAL FORMS OF THREE ECHINOIDS OF THE TORRES STRAIT REGION

NATURE AND SYSTEMATIC POSITION OF THE MATERIAL

All of the material considered in this paper was obtained in the Torres Strait region, Australia, in 1913. It consisted of the developing eggs and young larvæ of the echinoids *Echinometra mathœi* (de Blainville), *Peronella (Laganum) lesueuri* (Agassiz) and *Salmacis virgulata alexandri* (Bell).

Echinometra is a genus of the Family Echinometridæ, sub-order Camarodonta, order Centrechinoida; *Peronella* is a genus of the sub-order Clypeastrina, order Exoclycloida; *Salmacis* is a genus of the Family Temnopleuridæ, sub-order Camarodonta, order Centrechinoida.

H. L. Clark ('21) states that *Echinometra mathœi* "is probably the commonest sea-urchin in the world, as it ranges throughout the full extent of the vast Indo-Pacific region," and that *Peronella lesueuri* "occurs on the southern coast of Queensland and on the coast of West Australia as far south as Freemantle at least. It is common in the Aru Islands and northward through the East Indies to Hongkong and southern Japan. It is not known from the Pacific Ocean proper." The same author states concerning *Salmacis*, "Although typical examples of *alexandri* are easily distinguished from typical *virgulata*, there is no doubt that the two forms intergrade along the northeastern coast of Australia. True *virgulata* has been taken as far south as southern Queensland, while we found at Badu, on an eel-grass bottom, a small but typical specimen of *alexandri*, the form really characteristic of New South Wales."

The observations on the development of larval forms, presented in this paper, are based almost wholly on living material. For certain purposes, as will be noted later, larvæ were fixed in Flemming's stronger fluid, in picro-acetic acid or, for the preservation of skeletal structures, in alkaline alcohol after the larvæ had been killed in fresh water. By this method of killing, which was recommended to me by Dr. Caswell Grave, distortion of the form of the larvæ, which follows the use of the common killing fluids, is avoided. The method is admirable for the purpose for which it was devised, namely for the preservation of skeletal structures in their natural form and position. It does not and was not intended to give satisfactory preservation of the soft parts of the body.

EARLY DEVELOPMENT OF ECHINOMETRA MATHÆI

The work on *Echinometra mathœi* was done on the Island of Mer in the Murray Islands. On September 26, among the specimens collected was one which began to extrude eggs as soon as it was brought into the laboratory. Some of these eggs were fertilized immediately with active spermatozoa obtained by opening males in the usual way.

The eggs are small, heavily pigmented and opaque. It was therefore impossible to follow the phases of mitosis with any precision, and the first clear indication of division lay in the meridional constriction that marked the beginning of the separation of the first two blastomeres. This constriction appeared in one hour and five minutes after insemination. The actual process of migration of the primary mesenchyme cells into the blastocœle could not be observed because of the opacity of the egg, and it was not until just prior to the beginning of gastrulation that these cells could be seen distinctly. At that time the mesenchyme cells formed a very striking ring at the posterior end of the blastocœle. The anterior end of the blastula, and gastrula, has the form of a plate of elongated cells.

Fig. 1

Fig. 2

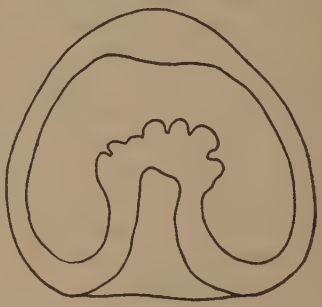

Fig. 3

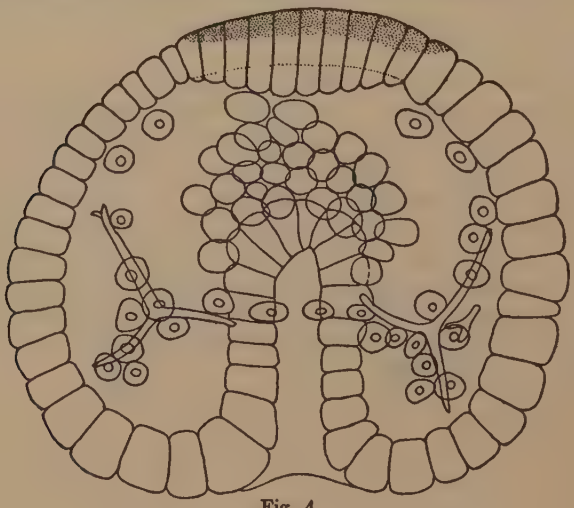

Fig. 4

The first skeletal spicules, of the usual tri-radiate form, were formed about 18 hours after the insemination of the egg. Gastrulation began within a few minutes after the appearance of the skeletal spicules. In the process of gastrulation the archenteron pushed through the ring of mesenchyme cells, the ring-like arrangement persisting even after the skeletal spicules had increased considerably in size. In this lot of larvæ the presence of multiple skeletal spicules was the rule rather than the exception (figs. 1 and 4). At 20 hours the skeletal spicules were well established and the secondary mesenchyme was arising from the inner end of the archenteron (fig. 4).

The form of the young pluteus is assumed during the second day, the skeleton of the post-oral and of the antero-lateral arms being well formed by the forty-fourth hour (fig. 5). The body skeleton forms a rather simple basket

Fig. 5

The recurrent rod is double, thus giving two openings, one of which is much larger than the other, at the sides of the body (figs. 5 and 6). The body rods are heavy and rather smooth. At their lower ends, where they come in contact with one another, they are broadened into a disk. The post-oral rods are fenestrated and at this stage distinctly constricted at their points of union between each fenestration (fig. 5). The ventral transverse rods and the connecting rods are rather heavy. At this stage the ventral transverse rods, but not the dorsal transverse rods, are broadened at their connecting ends.

The larvæ were very active, which made it difficult to get a complete camera sketch of the pluteus. The demands of other work stopped the attempt to get a complete camera drawing of the entire larva. Camera sketches of the skeleton in ventral view and in dorsal view, together with a

free-hand sketch in side view (fig. 6), were made. The two camera drawings have been combined in a composite sketch (fig. 5), which may not be more than approximately correct.

COMPARISON OF PLUTEUS OF MATHÆI WITH THOSE OF OTHER SPECIES OF ECHINOMETRA

This larva may be compared with that of *Echinometra lucunter*, 2 days old, described and figured by Mortensen (1921, fig. 25). The chief difference between the skeletons of the larvæ of the two species lies in the much greater thorniness of the *lucunter* larva.

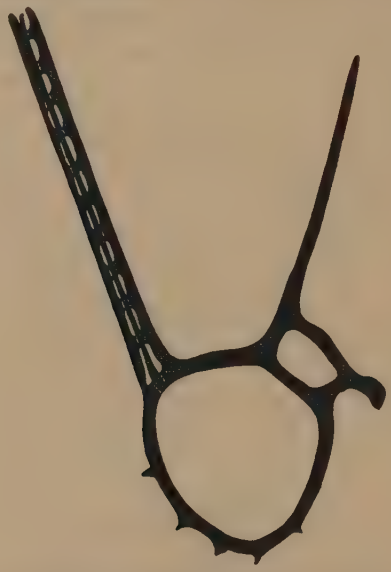

FIG. 6—Skeleton *Echinometra mathœi* 48 hours, side view.

In the 68-hour *mathœi* larva (fig. 7), all parts of the skeleton have become much heavier than they were at 44 hours, and the ends of the transverse rods connecting the recurrent rods have broadened into disks at their point of contact. Mortensen's material was not sufficiently preserved to enable him to give complete figures of the skeleton in the plutei of either *Echinometra oblonga* or *Echinometra mathœi*. For *oblonga* he noticed that the skeleton is not very thorny; that the recurrent rod is double, and that the body skeleton forms a basket structure.

Mortensen's notes on his living material made it possible for him to state that the body skeleton in *E. mathœi* "forms a basket structure, very thorny, and very oblique, the recurrent rod being considerably shorter than the body rod." He was not able to state whether the recurrent rod is double as in

E. oblonga and *E. lucunter*. He states with respect to the skeleton in the young pluteus of *E. oblonga*, "that the body skeleton forms a complicate basket structure, the recurrent rod being double; it is not very thorny." ('21, page 75.) His data therefore seem to make it clear that the pluteus that I have studied is not the same as the one studied by him. Mortensen's *E. mathæi* plutei had a very thorny body skeleton. The body skeleton in

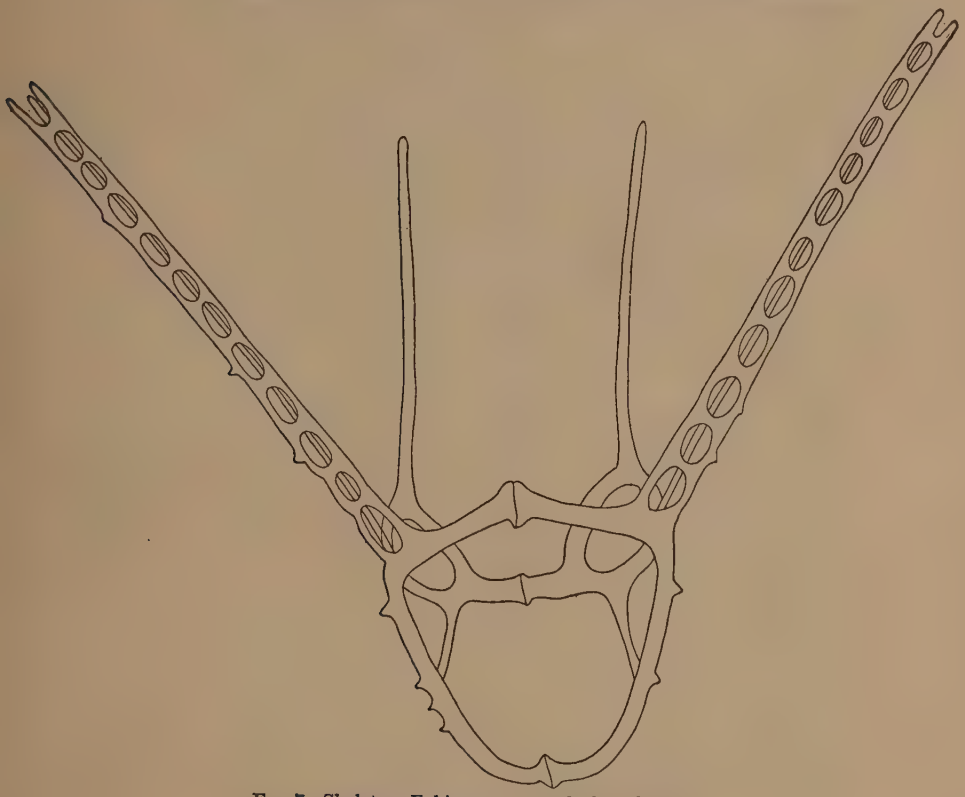

FIG. 7—Skeleton *Echinometra mathæi*, 68 hours, ventral view.

the form studied by me is smooth. It thus resembles that of the larva of *E. oblonga* described by Mortensen more closely than it does that of his *E. mathæi* pluteus. My specimens were identified for me by Dr. H. L. Clark, and *mathæi* is the only species described by Clark ('21) from the Murray Islands, or in fact from the Torres Strait region.

It is possible that the difference in thorniness between Mortensen's larvæ and those that I have studied may be due wholly to differences in the condition of the cultures. It is well known that slight changes in the environ-

mental medium may induce considerable changes in the character of the larval skeleton, but in view of the fact that "de Meijere and H. Lym. Clark are inclined to regard *Ech. oblonga* only as an extreme form of *Ech. mathœi*, while Doderlein makes it a type of a separate genus" (quotation from Mortensen '21, page 75), the differences in the larvæ may be a matter of some importance. If my observations on the skeleton of *E. mathœi* were in agreement with those of Mortensen, they would furnish support to this idea.

EARLY DEVELOPMENT OF PERONELLA LESUEURI

A provisional identification made for me in the field by Dr. H. L. Clark, of a Clypeastroid occurring in considerable abundance at Badu was that it was one of the Laganidæ and was either *Laganum* or *Peronella*. Throughout my notes on work with this form I designated it as *Laganum sp.*, and it was under this name, at my suggestion, that the results of an investigation of some of the material collected at Badu were published by Medes ('15). Shortly after the appearance of Miss Medes' paper, I received from Dr. Mortensen a reprint of his paper *On the Development of Some Japanese Echinoderms* published in December 1914, in Annotations Zoologicæ Japonensis, in which he gave a preliminary description of his work on *Laganum decagonale*.

Late in 1917 when I was working on the manuscript of this paper, Dr. Clark kindly sent me a positive identification of the forms that had been used in the work, and identified the clypeastroid in question as *Peronella lesueuri* (Agassiz). Further, Mortensen obtained from Dr. Clark a specimen of the Badu form and concluded, on comparing it with his Japanese specimens, that they were identical. (Mortensen '21, page 110.) Both Mortensen and I, then, worked with *Peronella lesueuri*.

It was a matter of some surprise to find that the eggs of *Peronella* were large and very opaque, and that they developed only to a pluteus-like form with two arms. Quite incidentally, as my investigations were in a somewhat different field, two stages of these larvæ were preserved, one of 29 hours in strong Flemming, for histological study, and one of 55 hours for the study of the skeleton, and a free-hand sketch of a 24-hour larva was made. The developing larvæ were so opaque that the details of the process of formation of the blastula and of the gastrula could not be followed with any degree of certainty.

The preserved material was given to Miss Medes for study and her investigations showed that the 29-hour larva contained a well-developed echinoid rudiment, and that the opening to the amnion was median and anterior (Medes '15).

Mortensen's ('21) study of a complete and well-preserved series of *Peronella* larvæ have enabled him to determine the manner of origin of the amnion in *Peronella*. It was impossible to do this in the single stage that Miss Medes had for investigation.

In my preliminary study of this form I drew a figure of a young pluteus through whose opaque walls the outlines of a stomach and œsophagus could be seen, and regarded the opening at the anterior end of the larva as the mouth. We continued to regard it as such until the matter of interpretation of sections arose. The sections convinced us that this opening really led into the amniotic cavity, and it seemed that I must have been mistaken in my identification of the mouth and digestive system during my study of the opaque living larva. Even though we had not questioned the accuracy of the sketch we should not have been able to advance the explanation of the formation of the amnion in *Peronella lesueuri* that Mortensen, from a complete set of material, has been able to establish beyond doubt. On the basis of the material at our command we had no evidence that the amnion had formed as a posterior prolongation from the pharynx. With Mortensen's evidence, however, there is no escape from his conclusion.

COMPARISON WITH OTHER WORK ON PERONELLA

A lack of agreement in the results of Medes and Mortensen, based on differences in observation, concerns the cœlom. Medes interpreted two spherical vesicles which lie one on each side of the fifth primary tentacle as probably representing the left posterior enterocœle (l. c. p. 139, and fig. 19, p. 136). She found no evidence of a right enterocœle. Mortensen ('21, p. 115) holds that there is here only a general body cavity and that a special homology between its different parts and the anterior and posterior enterocœles of typical echinoid larvæ can not be carried through. Miss Medes' conclusions regarding the complete independence of these two cavities from the anterior enterocœle were based on repeated studies of serial sections of plutei and the reconstruction of the pluteus, together with its cœlom, with wax plates. This matter, as well as that of the pore canal, should be reinvestigated fully before a positive conclusion is drawn.

EARLY DEVELOPMENT OF SALMACIS VIRGULATA ALEXANDRI

The early development of the pluteus of no species of the genus *Salmacis* was known at the time that this paper was prepared. Mortensen ('21) described three other Temnopleurid larvæ, the larvæ of *Temnopleurus toreumaticus*, *Temnotrema sculpta* and *Mespilia globulus*.

Salmacis virgulata alexandri (identified by H. L. Clark) was found in considerable abundance at Badu Island, on a grassy bottom. It was in excellent condition for embryological work during the first week of November 1913. In the first lot of eggs used, there were many immature ones. These were given off through the genital pores as freely as were the mature eggs. Not only were these eggs immature, as was evident from the fact that they were in the oöcyte stage, but they were much smaller than the mature eggs. The only male opened on this day furnished very active spermatozoa, and fertilization of the mature eggs was readily accomplished. The eggs

were transparent and the phases of nuclear division were followed readily.

Development was rapid. In one lot of eggs the mid-anaphase of the first cleavage was reached in 32 minutes after insemination. The second cleavage was completed 57 minutes after insemination. In the slowest lot of eggs the mid-anaphase of the first cleavage was reached 38 minutes after insemination. As no thermometer was available it is not possible to show that the difference in the length of the insemination-cleavage interval was associated with difference in temperature.

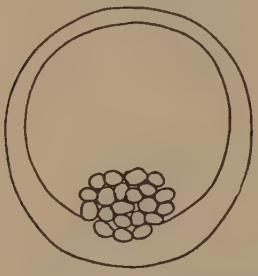

FIG. 8—*Salmacis*, mesenchyme formation.

FIG. 9—Skeleton of *Salmacis*, 29 hours, ventral view.

FIG. 10—*Salmacis* skeleton, 29 hours, side view.

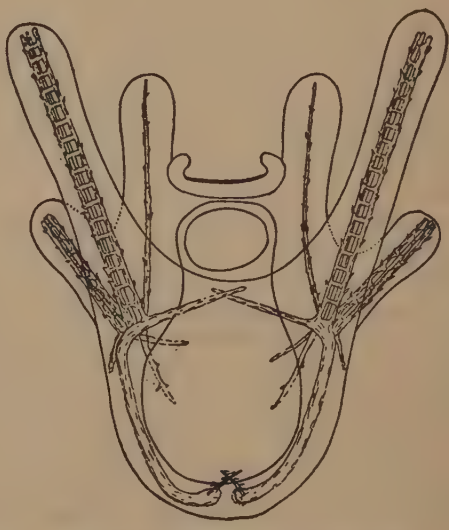

FIG. 11—*Salmacis* pluteus, 29 hours, ventral view.

Primary mesenchyme cells migrated from the posterior wall of the blastula into the blastocœle in five hours and 45 minutes (fig. 8), and gastrulation began in about nine hours after insemination. The stage of the pluteus was reached quickly, the post-oral, antero-lateral and postero-dorsal arms being well established, and the rudiments of the pre-oral arms having appeared 29 hours after insemination (figs. 9, 10 and 11). The post-oral, antero-lateral, postero-dorsal and body rods are exceedingly spiny.

FIG. 12—*Salmacis* pluteus, 55 hours, dorsal view.

The plutei 55 hours old (fig. 12) were vigorous and well formed. The skeletal rods of the arms and the body rods had increased in spininess. The dorsal arch and the pre-oral arm rods were well developed (figs. 13, 14 and 15).

COMPARISON OF PLUTEUS OF SALMACIS WITH PLUTEI OF OTHER TEMNOPLEURIDS

In two respects the pluteus was unlike any that I had seen before—the attainment of a stage in which four pairs of arms have appeared in so short a time and in the beautiful fineness of structure of its skeleton. The illustrations fail to indicate the real beauty of this framework. The deli-

cate spininess of the skeletal rods is so unusual that it was impossible not to doubt the accuracy of the sketches made during the study of the living larva and it has been reassuring to have perfectly preserved material for use in verification of the sketches made at the time of study.

Mortensen ('21 p. 207) has suggested that there may be a distinct larval type peculiar to the Temnopleurinæ. It is therefore interesting to be able to compare the *Salmacis* pluteus with the forms that Mortensen has described.

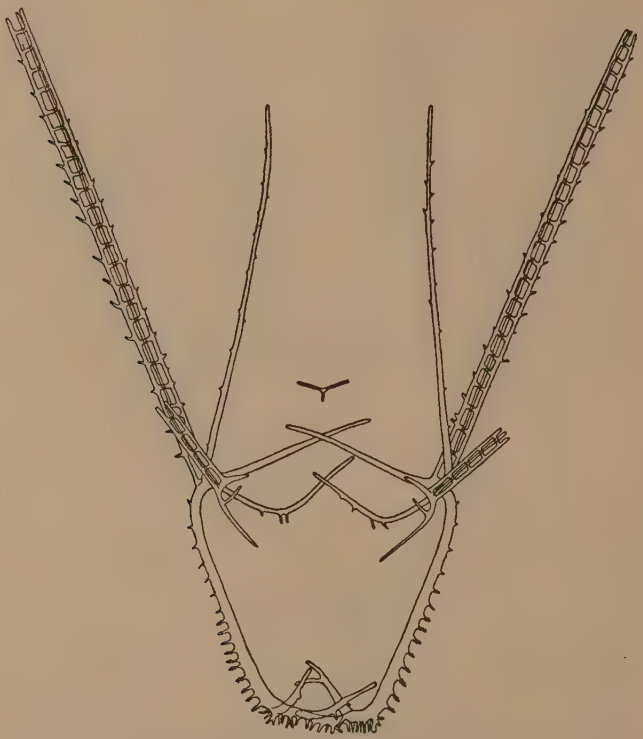

FIG. 13—Skeleton *Salmacis* pluteus, 53 hours, dorsal view.

Temnopleurus toreumaticus is similar to *Salmacis* in the rapidity of its early development. The side view of the skeletons of the plutei of the first stage show that the skeletons are similar in the termination of the body rods in two branches and in the angle formed between recurrent rods and antero-lateral rods. There is a general resemblance between the skeleton of Mortensen's 9-day pluteus of *Temnopleurus* and the 55-hour pluteus of *Salmacis*.

In Mortensen's *Temnotrema* sculpta larva, 2 1/2 days old, there again is close similarity between the side view of the skeleton of this larva and that of the *Salmacis* pluteus of 29 hours. There is a general similarity in the outline of Mortensen's pluteus ('21, plate VI, 4), 18 days old, and my 55-hour larvæ. The chief difference is in the lack of pre-oral arms in the *Temnotrema* larva. It is possible, even probable, that in both the *Temnopleurus* and *Temnotrema* larvæ described by Mortensen, developmental proc-

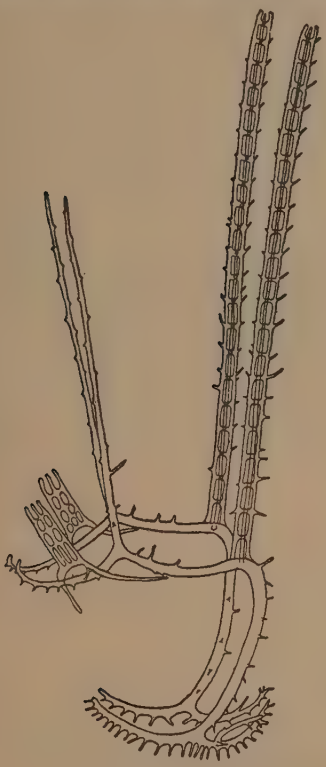

FIG. 14—Skeleton of *Salmacis* pluteus, 53 hours, side view.

FIG. 15—Skeleton of *Salmacis* pluteus, 55 hours, from right side of larva.

esses had stopped some time before the ages noted had been reached. It has been my experience with laboratory cultures of echino-plutei that the larvæ may develop progressively for three days and then remain in the characteristic third-day stage for a prolonged period unless a sufficient quantity of suitable food is provided. Resorption of the skeletal rods is apparently coincident with starvation. It is therefore possible in laboratory cultures to have plutei two weeks old, which have been able to obtain food enough to keep themselves alive, but not enough to enable them to develop

beyond a third or a fifth day stage. It is not certain that food is the only determining factor. An occasional culture is obtained that is far better than the average in its possibilities for successful development. The conclusion to this effect, reached in a study of the variation of *Lytechinus* plutei under laboratory conditions (Tennent '10, p. 663), has been confirmed by more recent observations many times. The matter is possibly one of an occasional perfect fertilization reaction—perfect because the eggs and spermatozoa have been in the best physiological condition to enter into that reaction. It may be a matter of nutrition, *i.e.* some lots of eggs may be richer in food materials than others; or there may be a combination of these two conditions. In any event the fact remains that, given cultures of the same age that have shown an equally high degree of membrane formation following insemination, by the third day it is possible to conclude whether any of these cultures have good chances of producing a high percentage of advanced plutei.

From the evidence at hand it seems probable that Mortensen is correct in the suggestion that his "Echinopluteus of Temnopleurid (?) Species a" ('21, pp. 57 and 58) is a *Salmacis* larva. While my *Salmacis* larvæ have not developed to the stage at which a transverse rod is present, the rather characteristic conformation of ventral transverse rods, dorsal transverse rods and the fine thorniness of the antero-lateral rods, suggest the close relationship of the two forms. Similarly Mortensen's figure of the "Echinopluteus of Temnopleurid (?) Species c" ('21, plate XI, fig. 3) is like that of my figure of the 55-hour *Salmacis* larva (fig. 12).

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IV

ON THE POSTLARVAL DEVELOPMENT OF THE CORAL MÆANDRA AREOLATA (L.)

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Nine text-figures

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ON THE POSTLARVAL DEVELOPMENT OF THE CORAL MÆANDRA AREOLATA (L.)

There are rather few data on the development of the Madreporaria. De Lacaze-Duthiers (1873, 1894, 1897) and von Koch (1882, 1897) studied the development of a number of Mediterranean corals, none of which belonged to the true reef-corals. The only reef-coral of which the development of the young polyps is known in full detail is *Siderastrea radians* (Duerden, 1904). The same author (Duerden, 1902) described a few stages of the development of some other West Indian corals, viz., *Agaricia agaricites, Mussa (Isophyllia) dipsacea, Favia fragum* and *Mœandra (Manicina) areolata.* The development of the larvæ and young polyps of the Bermudian form *Agaricia fragilis* is made known by Mavor (1915).

As for *Mœandra (Manicina) areolata,* besides the data given by Duerden (1902), the larval development of this species forms the subject of an extensive study by Wilson (1888). In this paper, however, no observations are made on the postlarval stages.

During the six weeks in July and August 1925, in which I studied at the Laboratory of the Carnegie Institution of Washington at Tortugas, I was using, among other species, the coral *Mœandra areolata* for researches on the food of reef-corals. At a certain period many of the colonies contained ripe larvæ and I resolved to rear these for the study of their development. The results are found in the following pages. I wish to express my sincere thanks to the Carnegie Institution of Washington and to Dr. W. H. Longley for aiding me with the needed facilities.

MATERIAL AND METHOD

On July 19 and following days a great number of the colonies of *Mœandra areolata* which were brought from the reefs into the laboratory were found to contain numerous larvæ. These were visible through the tissues of the polyps, especially in expanded tentacles, where they were constantly moving. Duerden (1904) observed a similar phenomenon in *Siderastrea radians.* Here it was impossible to make out whether the swimming of the larvæ in the gastric cavity was due to their own action or to movements of the cilia of the gastric cavity. In *Mœandra* the larvæ swim through their own ciliary action; often two or more larvæ could be seen moving in the same tentacle while their movements took place in quite different directions. The larvæ gradually hatched through the mouths of the polyps, often after a sudden jerk of the oral surface of the whole colony.

I kept these larvæ in large glass vessels in which they remained moving for two or three days. After this time a great number of larvæ became attached to the sides of the vessels and on slides which I had put on the bottom and against the sides of the vessels for this purpose. The larvæ on the slides formed the material on which the following observations are based.

The larvæ attached to the slides could be studied under the microscope as well from the oral as from the aboral surface in their subsequent stages of growth. Moreover, the larvæ being attached to the slides with straight ridges, the dorsal and ventral region of the polyps, which was obvious in the first stages, was also known in the later stages, owing to the position of the young corals on the slides. So the drawings made from different stages of the same polyp could be compared in the same position. The young corals were kept on the top of small bottles placed in a tank with running sea-water. A few lumps of the skeleton of dead coral-colonies were put at the bottom of the tank to make sure that the sea-water contained carbonate of lime in sufficient quantity.

DEVELOPMENT OF THE MESENTERIES

Shortly after attachment the young polyps usually have three complete pairs and one incomplete pair of mesenteries (figs. 2*a*, 4). In some of them, however, only two pairs of mesenteries are complete, the third and fourth incomplete (fig. 4, *g*). In the following part of this article the word pair is used for two mesenteries found on the left and the right of the stomodæum, originating at about the same time and at the same distance from the dorso-ventral plane. The word pair is, therefore, used here in a different sense from that used by O. and R. Hertwig (1879), who denote as a pair of mesenteries (Septenpaar) the two mesenteries between which an endocœl is enclosed. Concerning the order of appearance of the mesenteries in *Mœandra areolata* I can state only that the numbers 3 and 4 (cf. fig. 2*a*) are correct, for in all of my young attached polyps the mesenteries numbered 1 and 2 had already reached the stomodæum. Wilson (1888) has stated that in *Mœandra areolata* the order of appearance of the first two pairs of mesenteries is as indicated in figure 2*a*. Moreover, in all other young polyps of Madreporaria studied by Duerden (1902), the first two pairs of mesenteries appeared in the same way. Of the third and fourth pair, however, the latter remains incomplete for a considerable time, while the third pair soon reaches the stomodæum. The young polyp now has reached the Edwardsia-stage. In general the order of appearance of the mesenteries till this stage is quite different in the various forms of actinians of which the development is known (cf. Pax, 1914). In Madreporaria this appearance seems to take place constantly in the same manner as indicated by Duerden (1902) and as in figure 2*a*. This order of appearance is in accordance with that found by McMurrich (1891) in the actinians *Aulactinia* and *Rhodactis*.

The appearance of the mesenteries of the fifth and sixth pair also is in accordance with the order found by Duerden (1902). According to Wilson (1888) and Mavor (1915, *Agaricia*) the fifth pair of mesenteries develops between the first and third, the sixth pair between the first and second. In my polyps the pair between the first and the second developed before that between the first and the third (cf. fig. 2*b*, where only one mesentery of the sixth pair is visible, while both mesenteries of the fifth pair are present, and fig. 4, *d*, *f*, *h*, and *i*, in which the mesenteries of the sixth pair are absent or present at one side of the polyp only). Although in my material as a general rule the mesenteries numbered 5 in figure 2*d* appeared before those numbered 6, they develop almost simultaneously. It is therefore of little importance that the data in the literature differ somewhat on the order of appearance of these mesenteries.

One or two days after attachment in this way the polyp has six pairs of mesenteries, three pairs of which reach the stomodæum. The polyps now have reached the protocnemic stage (Duerden) or Halcampula-stage (cf. Carlgren, 1906; Pax, 1914, 1925). As for the mesenteries this stage remains practically unaltered for a considerable time. The mesenteries enlarge toward the stomodæum, those of the fourth pair reach it at about five days after the attachment of the larva (fig. 1*b*); the mesenteries of the fifth and the sixth pair remain incomplete for a longer time. In the second week after attachment these mesenteries also become complete and the young polyp has twelve mesenteries distending from the stomodæum to the margin (figs. 1*c*, 6). I have made no observations on the development of the mesenteries of the second cycle (the metacnemes), as these were not distinctly visible, even in the oldest stages of my material.

DEVELOPMENT OF THE TENTACLES

In the later half of the first week after attachment the young polyps show the first traces of tentacles. These appear as white knobs above the exocœles (fig. 1*b*) and are clearly visible by their colorless extremity. Here a number of nematocysts is found and this part is devoid of zooxanthellæ, while these algæ are present in great numbers in the basal part of the tentacles and along the mesenteries. These six tentacles (the first cycle of exotentacles) appear simultaneously and have approximately the same size.

In the second week after attachment three more tentacles make their appearance. In some of the larvæ they are already to be seen in the end of the first week, but as a rule they do not grow out before eight or nine days after attachment. As already stated, the dorso-ventral plane, which could be determined in the young stages, remained known by the fact that the young corals were fixed on the slides in a definite place. In *Mœandra areolata* the first three members of the first cycle of endotentacles appear

invariably in the ventral endocœl and in the endocœls between the mesenteries 2 and 5 (figs. 1*c*, 6). Soon after these tentacles are visible a dark spot is to be found at the corresponding places in the dorsal endocœl and those between the mesenteries 1 and 6 of each side. This is due to a considerable amount of zooxanthellæ. It requires several days before tentacles develop here also; in general after twelve to sixteen days after attachment of the larvæ, the first cycle of endotentacles is complete. For some days this cycle is composed of three larger and three smaller ones, but toward the middle of the third week after attachment the endotentacles have approximately the same size (fig. 1*d*). The polyp possesses now six larger exotentacles and six endotentacles of smaller size. In the development of the tentacles, as well as in that of the mesenteries, there is a great deal of individual variation in different polyps. Some individuals are growing much faster than others, as may be seen in figures 4, 5 and 6, which represent a colony of polyps of the same age.

As soon as the tentacles have appeared the young polyps are capable of contraction and expansion. When undisturbed they are fully expanded, but they contract suddenly after a gentle touch or a shock of the surrounding water. The oral disk then contracts over the stomodæum and the tentacles, completely covering those organs. Especially the larger polyps (figs. 1*d*, 6) suddenly contract on a slight stimulus and only very gradually expand again to their full size. These larger polyps will feed readily on small morsels of meat of mollusks.

THE SKELETON

The development of the skeleton of the young corals begins at a very early stage. One day after attachment the first traces of the basal plate are found as a thin irregular calcareous layer secreted at the base of the polyp. It distends somewhat farther than the soft parts at the base and is found projecting somewhat outside the tissues; figure 1*a* is a drawing of a young polyp in which it is represented; in most of the other figures the basal plate has been omitted. About four days after attachment a narrow erect ridge appears which surrounds the basal part of the polyp (figs. 1*b*, 2*e*). This is the beginning of the theca which gradually grows higher, the corallum thereby becoming more or less cup-shaped (figs. 3*c*, *d*).

Soon after the attachment of the larvæ the septa appear also, those of the first cycle in the endocœls, as small irregular corpuscles which as yet are not united in definite ridges (fig. 2*c*). Two or three days after attachment the first and the second cycle of septa are to be seen, those of the second cycle in the exocœls, thereby corresponding in place with the six exotentacles (fig. 2*d*). The septa of the first cycle now are much more strongly developed than those of the second cycle and they remain more conspicuous for a long period. About four or five days after attachment of the larva the septa are

definitely shaped as ridges and have lost their loose structure of small corpuscles (figs. 2*e*, 3*a*). Usually about seven to ten days after attachment the septa of the third cycle develop also. They consist of small corpuscles on the theca with very little prominence (figs. 2*f*, 3*b*). In older stages they are often not distinctly seen, as the basal plate has become thicker and the septa of the third cycle retain their original strength for a considerable time. In the various individuals in my material the development of the septa of the third cycle took place in a different manner; in some corals they were visible at a very young stage, in others they had not yet appeared at a much older stage.

At first the septa remain quite separated from the theca. Gradually they grow out toward the periphery, and ten or twelve days after the attachment of the larva they have fused with the theca. At about the same time the septa of the first cycle have enlarged toward the center of the corallum, where they fuse and form a part of the columella. Another part of the columella is formed by small excrescences arising from the central part of the basal plate.

A further peculiarity in the development of the skeleton is that in the third week after the attachment of the larvæ the septa of the second cycle fuse with those of the first cycle in a characteristic manner, by means of which the corallum obtains a strictly bilateral form (figs. 8, 9). The two septa of the second cycle from each side of the dorsal septum of the first cycle unite with the latter. Each of the two remaining septa of the second cycle of each half unites with one of the two lateral septa of the first cycle. The ventral septum of the first cycle remains free. The septa of the second cycle now have a curved course and the corallum possesses six groups of septa; a group of three at the dorsal side, two groups of two in each lateral part, and the free ventral septum. The corallum now has a striking resemblance with the stage in the development of *Siderastrea* represented diagrammatically by Duerden (1904) on page 89 (fig. 9*f*).

As I had to leave the Laboratory at Tortugas twenty days after I had begun to rear the larvæ of *Mœandra areolata* I could not make observations on older stages. The skeletons of a number of young polyps were preserved after the polyps had been killed, according to Dr. Grave's method, in distilled water with $MgCO_3$. As soon as the soft parts were gone the skeletons were mounted on slides. From such preparations figures 3 and 8 are made; all other figures are drawn from the living animals. In comparison with the larvæ reared by Duerden (1902) it may be noted that the young polyps of my material developed rather quickly. In Duerden's young polyps of *Mœandra areolata* of twenty-one days, represented on plate XIX, figs. 135 and 136, the septa of the second cycle have not yet developed, though one of the polyps has already twelve tentacles.

AGGREGATED COLONIES

Owing to the fact that a great number of larvæ were kept in comparatively small glass vessels, many of them settled in the close neighborhood of other larvæ on the slides. This resulted in the formation of aggregated colonies as described by Duerden (1904) in *Siderastrea*. Figures 4 to 8 are drawn from successive stages in the development of such a number of young polyps. Shortly after attachment to the slide the nine individuals were as yet not in close contact with one another (fig. 4). One day later (fig. 5) the size of the polyps was much larger, but one of them (*g*) had retained about the same size for want of space. Soon afterward this polyp died and the soft parts of the neighboring polyps (*f* and *i*) spread over the place where the polyp (*g*) had vanished (fig. 6). At this stage the theca has been formed at the margin of the polyps; between the fused polyps it is present as a small ridge, not thicker than that on the free outside. Between the polyps *f* and *i* the theca remains incomplete, as in older stages also (fig. 8). With the exception of this lone instance the individuals forming the aggregated colonies always remain separated from one another by a distinct theca. This probably would have also remained in the further development of the colonies. The shape of the corallum of the individuals of these aggregated colonies differs somewhat from the normal circular form, their thecæ presenting straight lines and sharp angles at the places where they have fused. The soft parts do not differ from those in normal simple polyps; the mesenteries and the tentacles have the regular number and arrangement. Also the septa present only slight differences from those in simple coralla (figs. 7, 8). The only abnormality is that one tentacle of the polyp *i* has two knobs.

This fusion of so many individuals into aggregated colonies in all probability is a very exceptional phenomenon, only possible in cases as in the laboratory when a great crowd of larvæ has to settle in a small vessel. In nature, however, occasionally adult coralla are found which are composed of two fused colonies. Each of the two colonies then forms a separate entity though the skeleton is fused with that of the other colony; at the oral surface they are always separated by a thin ridge which represents the fused thecæ of the two originally simple polyps which have united. Examples of such fused double colonies are fairly common on the reefs around Tortugas. The corallum of *Mœandra areolata* figured by Wilson (1888) on plate 1 is another instance of a colony composed of two parts, each evidently originated from a simple polyp. Here also the theca between the two parts is very distinctly visible as a thin ridge.

ZOOXANTHELLÆ

The newly hatched planulæ contain a great number of zooxanthellæ which they obtain while inside the gastric cavity of the mother-polyp. Wilson (1888) found that in *Mœandra areolata* the stages before the planula are

still devoid of zooxanthellæ. He found these algæ for the first time in the ectoderm cells of the planula, from there they migrate to the entoderm, their definite place. The brown color of the planulæ has different shades of intensity in the various individuals, owing to the quantity of zooxanthellæ in the tissues; some have a light brown hue, others are darker (fig. 4). These darker planulæ give rise to darker polyps than the lighter ones, as the zooxanthellæ seem to multiply with the same intensity during growth. In the young polyps the greatest quantity of zooxanthellæ is found in a circular area at about the middle of the oral surface, and around the stomodæum (figs. 1*a*, 4, 5). After some time the tentacles are developing in this circular area and the algæ are found in large numbers in the tentacles, especially near the tips. They are found also rather abundantly in the mesenteries (figs. 1, 6).

CONCLUSIONS

The development of the endotentacles which appear constantly in two successive groups of three resembles in some way the facts recorded by de Lacaze Duthiers (1872) in *Actinia mesembryanthemum*, some stages of which show a marked tri-radial arrangement of the tentacles (cf. fig. 31 of the cited article). The data in the literature on the development of other coral polyps seem to prove that this successive development of the endotentacles in two groups is an exceptional case (*Favia fragum*, Duerden, 1902; *Siderastrea radians*, Duerden, 1904; *Astroides calycularis*, de Lacaze-Duthiers, 1873; *Caryophyllia cyathus*, de Lacaze-Duthiers, 1897).

The bilateral arrangement of the septa in the oldest stages of my material (figs. 8, 9) is in accordance with that found by Duerden (1904) in *Siderastrea*. The same evidently occurs in *Agaricia* in which Mavor (1915) found the first stages of such an arrangement. Probably in older stages of *Agaricia* the bilateral symmetry will become still more pronounced by fusion of the septa of the second cycle with those of the first cycle. Sometimes in the development of actinians also a pronounced bilaterality occurs, *e.g.* in *Cribrina gemmacea* (cf. Carlgren, 1904). This phenomenon can be compared in some way with the peculiar arrangement of the septa as drawn in the diagrams of figure 9.

As in the majority of corals in which the young stages are known the septa in *Mœandra* develop in two cycles, first the six endosepta and soon afterward the six exosepta. This is in accordance with the facts described by Duerden (1904) in *Siderastrea* and by de Lacaze-Duthiers (1894, 1897) in *Flabellum* and in different species of *Caryophyllia*. The same occurs also in *Fungia* (Boschma, 1923). On the other hand in *Astroides* (de Lacaze-Duthiers, 1873, von Koch, 1882) the first twelve septa develop about simultaneously. This phenomenon is also found in *Balanophyllia* and seems to occur as a rule also in other Eupsammid corals (de Lacaze-Duthiers, 1897).

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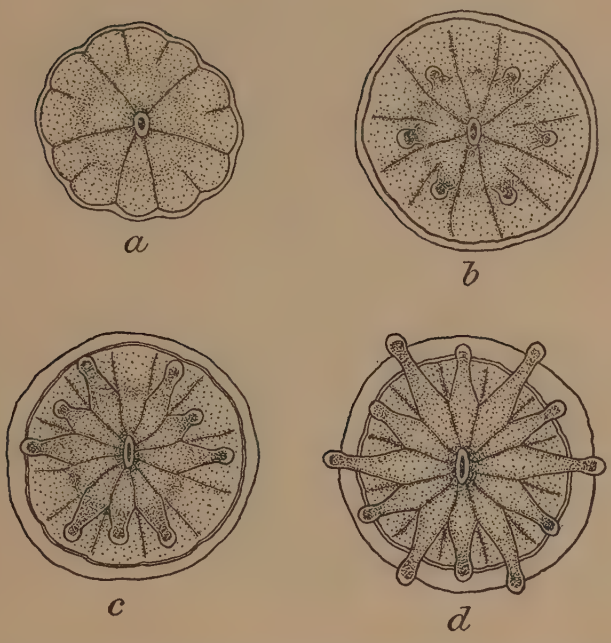

FIG. 1—Oral surface of young polyps, showing development of first and second cycle of tentacles. Zooxanthellæ are indicated by dots. Ventral region of polyps is directed toward lower part of figures. × 35.

a. Young polyp, one day after attachment, with six complete mesenteries. Basal plate is visible at margin.

b. The same, 5 days after attachment. Six tentacles of first cycle and first traces of theca are present.

c. Another polyp, 12 days after attachment. First three tentacles of second cycle have developed.

d. A third polyp, 18 days after attachment. Second cycle of tentacles is complete, the theca is already more or less cup-shaped.

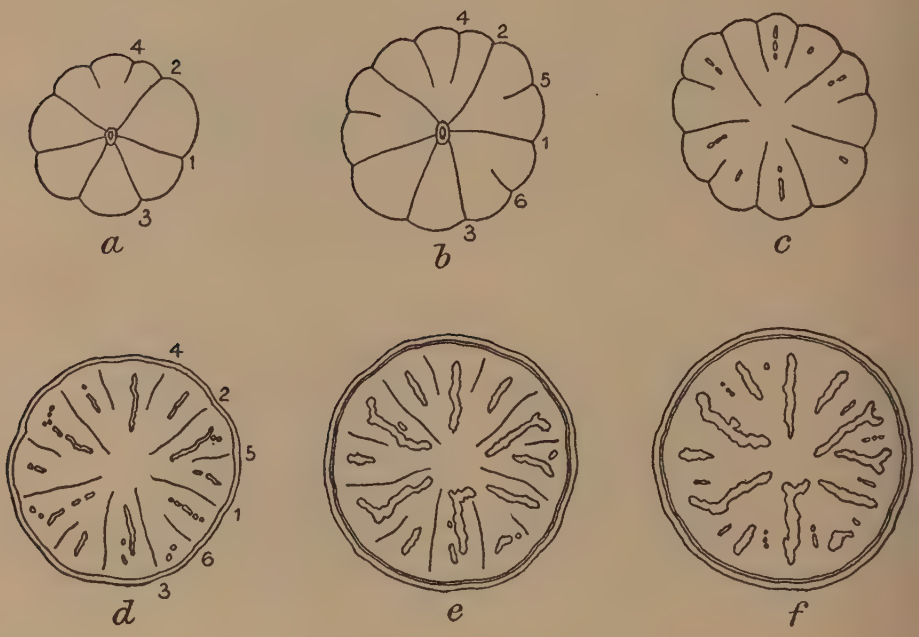

FIG. 2—Different stages of development of a young polyp, showing growth of septa. *a* and *b*, oral surface; *c-f*, aboral surface. Numbers placed near mesenteries indicate order of appearance. Ventral region of polyps is directed toward lower part of figures. × 35.

a, shortly after attachment; *b*, one day later than *a*; *c*, aboral surface of *b*, with first traces of septa; *d*, 2 days later than *a*, septa of first cycle are more prominent than those of second cycle; *e*, 4 days later than *a*, with traces of septa of third cycle.

Irregular corpuscles forming columella are not drawn. Basal plate is visible outside theca. Latter is quite distinct in *e* and *f*.

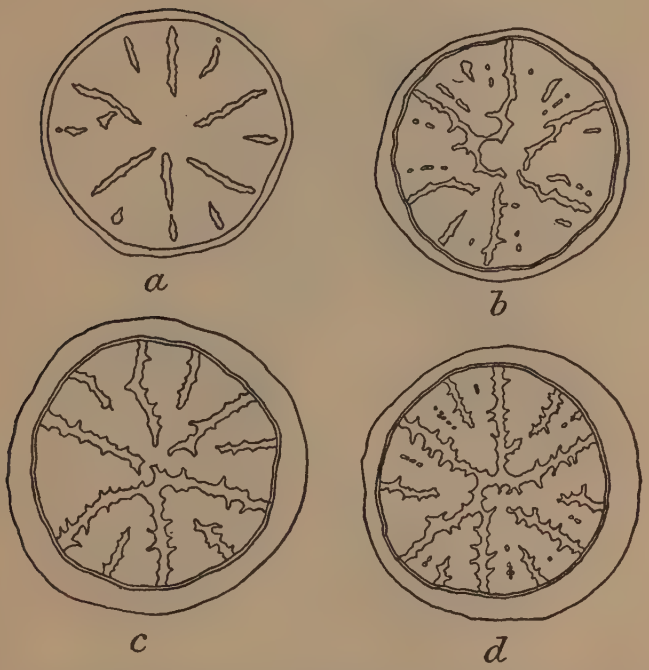

FIG. 3—Oral surface of skeleton of four different polyps after removal of soft parts. Ventral region of polyps is directed toward lower part of figures. × 35.

a, 5 days after attachment; two complete cycles of septa.

b, 10 days after attachment; first cycle of septa complete, second and third cycle incomplete.

c. 10 days after attachment; first cycle of septa complete, second cycle nearly complete, no septa of third cycle. Theca is strongly developed.

d, 17 days after attachment; 2 complete cycles of septa and an incomplete third cycle.

In *b*, *c* and *d* central parts of many septa have united to form a great part of the columella.

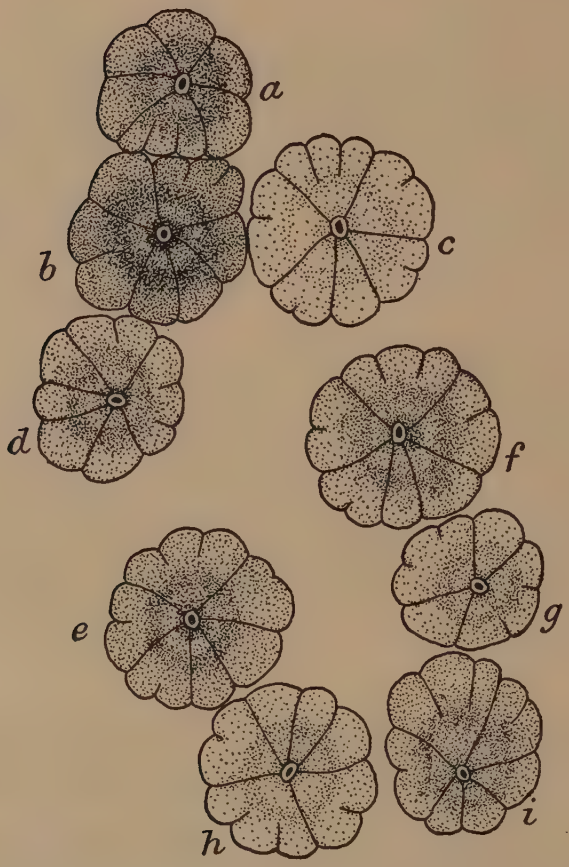

FIG. 4—A group of young polyps attached to a slide, shortly after attachment. Oral surface. × 35.

The letters indicate different individuals (same as in following figures). Zooxanthellæ are represented by dots.

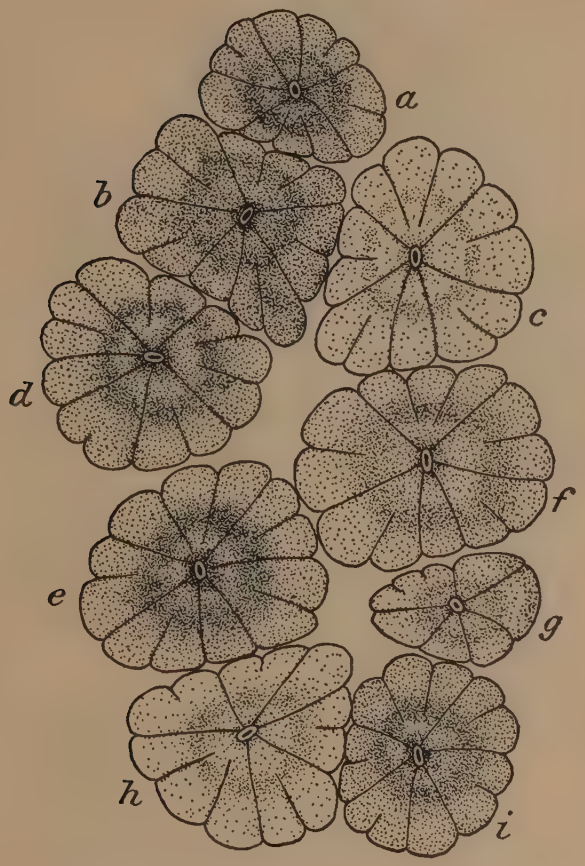

FIG. 5—Same polyps, about one day after attachment. Oral surface. × 35. The polyp *g* has developed little.

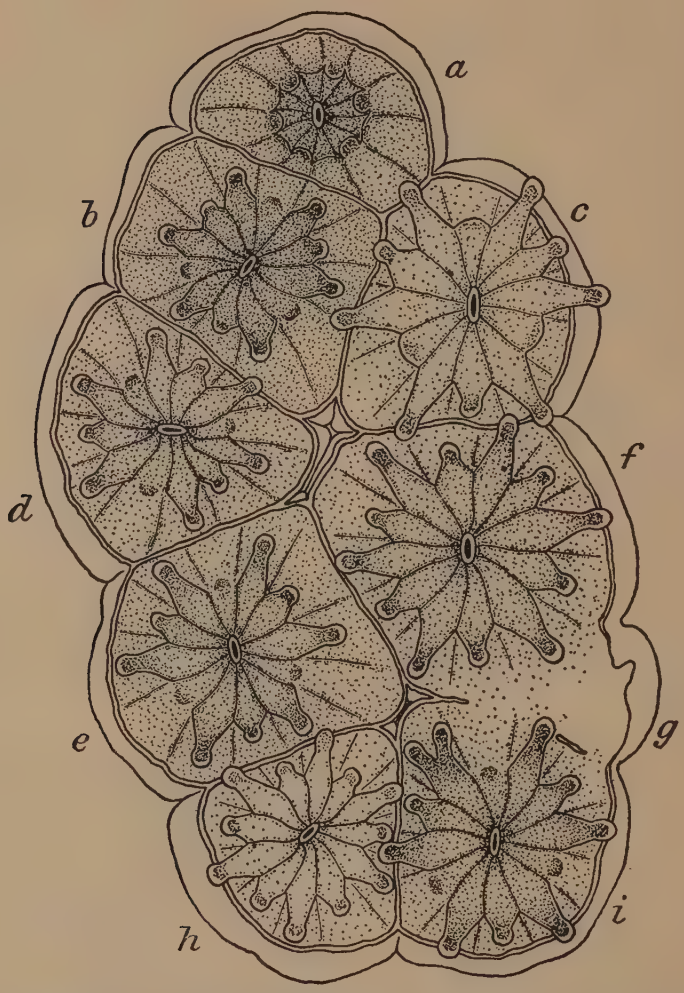

FIG. 6—Same polyps, 12 days after attachment. Oral surface. × 35. Polyp *g* has vanished, only parts of theca remaining. One of tentacles of *i* has two knobs.

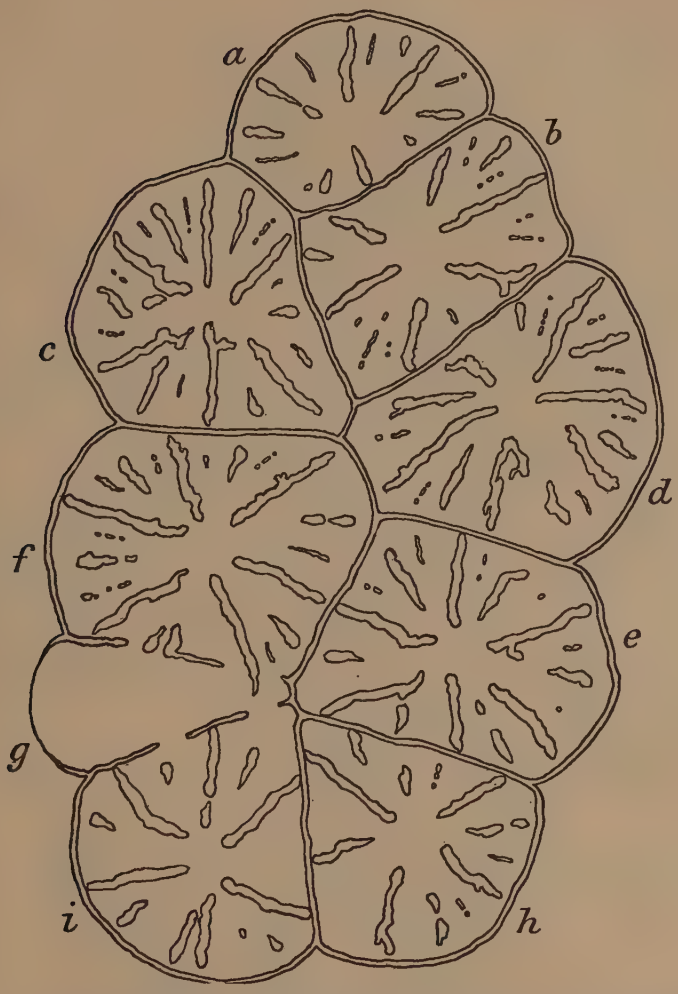

FIG. 7—Same polyps, 12 days after attachment. Aboral surface. × 35. Figure shows basal parts of thecæ and septa. Basal plate and columella, which was not distinctly visible, omitted.

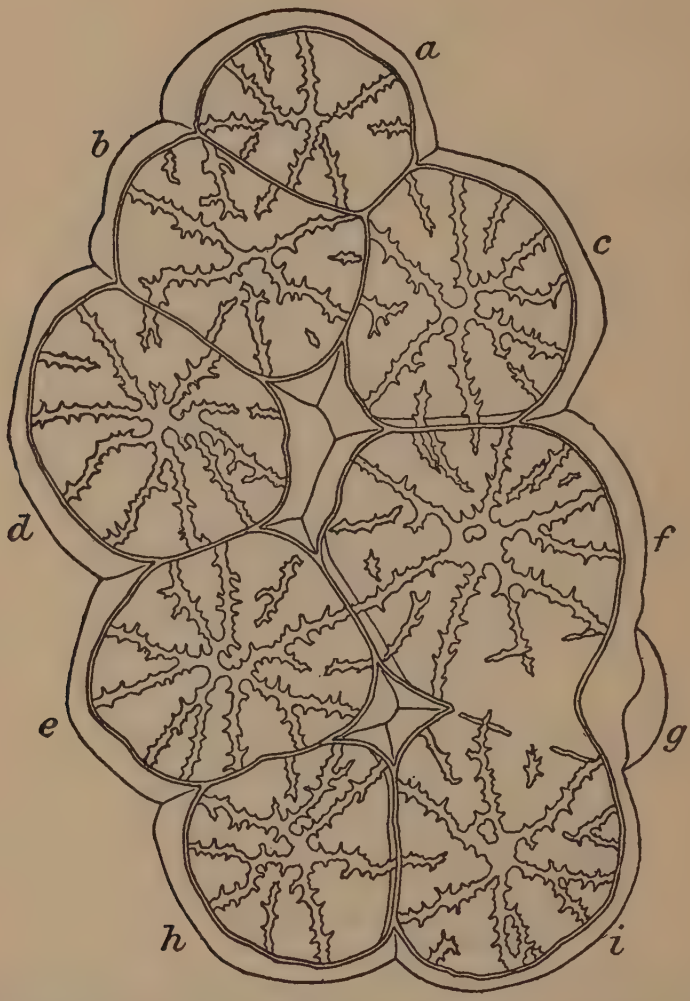

FIG. 8—Skeleton of same polyps, 17 days after attachment. Oral surface. × 35.

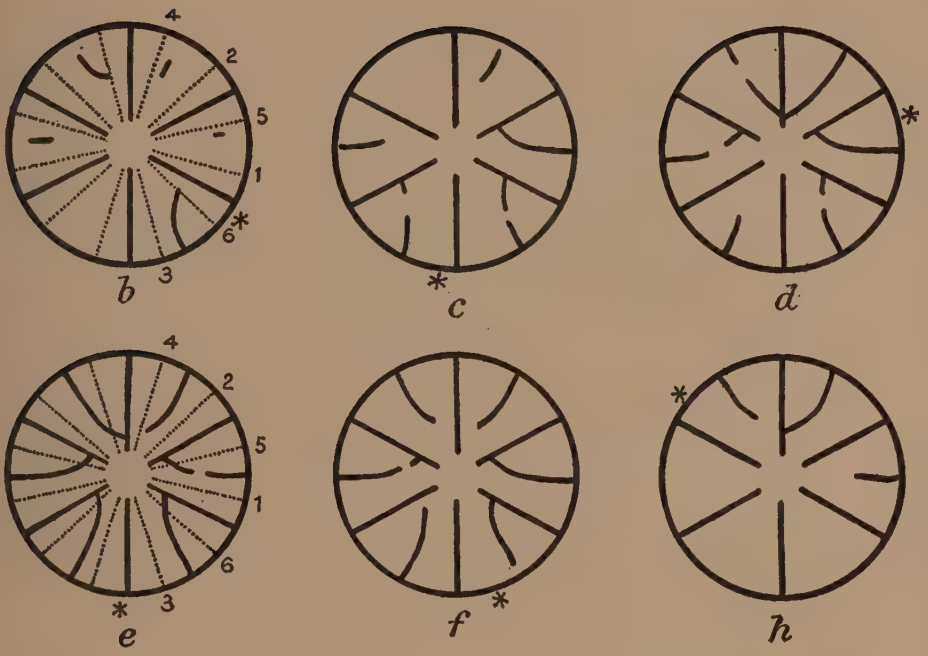

FIG. 9—Diagrams of polyps *b*, *c*, *d*, *e*, *f* and *h* of previous figure. Thecæ and septa are drawn with full line, in *b* and *e* the mesenteries are represented as dotted lines (numbers as in fig. 2). Asterisks are placed at spots which occupy lower parts of polyps in figure 8.

V

ON THE MORPHOLOGY, COLORATION AND BEHAVIOR OF SEVENTY TELEOSTEAN FISHES OF TORTUGAS, FLORIDA

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Four plates

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ON THE MORPHOLOGY, COLORATION AND BEHAVIOR OF SEVENTY TELEOSTEAN FISHES OF TORTUGAS, FLORIDA

INTRODUCTION

Parts of the summers of the years 1912 to 1915 were spent at the Marine Biological Laboratory of the Carnegie Institution of Washington at Tortugas, Florida, by invitation of the Director, the late Dr. Alfred G. Mayor. My particular purpose was to study the structure of the reproductive organs, the breeding habits and the embryology of whatever sharks and rays, elasmobranch fishes, I could get. As it turned out, Tortugas, lacking garbage, the refuse of slaughter houses and dead animals cast overboard, is not a very good place for sharks. These were studied mainly at Key West, where they are much more abundant and where, through the courtesy of Dr. Mayor, I fished for sharks whenever the *Dohrn* went for supplies, and at other times when she could be spared to make the trip. Lacking sharks at Tortugas, I turned to the teleosts, the bony fishes, and this paper embodies the general results obtained. Particular search was made for and especial attention given to the unusual forms.

Preliminary reports on the results of all this work have appeared in the Year Books of the Carnegie Institution of Washington for 1912 to 1915. Various articles have been published by the Carnegie Institution of Washington in the Tortugas volumes, and others have appeared elsewhere. A complete list of these papers up to the year 1924 may be found in the Mayor Memorial volume (Vol. XIX of Papers from the Department of Marine Biology of the Carnegie Institution of Washington, p. 83).

In all this work I had the cordial support of my late chief, Dr. Alfred Goldsborough Mayor, whose friendly advice and active help I shall never forget. For assistance in collecting the fishes, for hints as to their common names and their habits, and for many other kindnesses I am under obligation to our chief engineer, John Mills, and his willing assistants, George Tracy, Wm. Lee Wilson and Walter Mills.

The Tortugas archipelago or atoll is situated on the extreme outer end of the Florida submarine plateau, 70 miles due west of Key West. It consists of a group of seven small treeless islands, composed of coral rock and sand heaped up by the waves and wind, which with their surrounding reefs enclose a shallow lagoon of some 40 or 50 square miles' area. Only two of these islands are habitable and inhabited: Garden Key, near the center and completely covered over by Fort Jefferson, now deserted, and Logger-

head Key at the extreme west. This latter island is in the form of a flat crescent, concave side westward, having Tortugas Light near the center and the Laboratory toward the northern horn.

Except on the side extending toward Key West, the Tortugas atoll is surrounded by "blue water," and channels of such deep water penetrate irregularly between the islands. The Gulf Stream is only about 25 miles away to the south, and at times the winds bring its surface waters laden with gulfweed into the group. The temperature of the water in and around the Tortugas lagoon during the summer averages from 80° to 85° F. Here is to be found such a combination of warm shallow lagoon water, deep water and Gulf Stream water as to permit the development of an extremely large and varied fish fauna. In 1905 according to David Starr Jordan, 218 fishes had been recorded from the Tortugas. Several are added in this paper, and it is certain that a thorough investigation of the fish fauna of the Tortugas, carried on for a period long enough to make it really exhaustive, would largely add to the list of known fishes.

On reaching Tortugas early in June 1912, I found a fish fauna totally new to me and totally different from that which I had studied at Beaufort, North Carolina—one, in fact, which contained (so far as they came into my hands) not more than three species of fishes that I had ever seen before. Furthermore, only two papers have been published dealing specifically with Tortugas fishes. These are:

Jordan, David Starr. Notes on Fishes Collected in the Tortugas Archipelago [by Dr. Joseph C. Thompson]. Bull. U. S. Fish Commission for 1902. 1904, vol. 22, pp. 539-544, 2 pls.

Jordan, D. S., and J. C. Thompson. The Fish Fauna of the Tortugas Archipelago. Bull. U. S. Bureau of Fisheries for 1904. 1905, vol. 24, pp. 229-256, 6 figs.

These two papers are faunal lists with brief descriptions of particular specimens collected by Dr. Thompson and are devoid of keys, hence they could not be used to run down the fishes caught. However, the fish fauna of the Tortugas and the Florida Keys in general is thoroughly West Indian, so I fell back on the best book on West Indian fishes—

Evermann, B. W., and M. C. Marsh. The Fishes of Porto Rico. Bull. U. S. Fish Comm. for 1900. 1902, vol. 20, pt. 1, pp. 51-350, 112 text-figs., and 49 col. pls.

This was used constantly and was a great help. However, for fishes not in the Porto Rico report, and as a court of last resort in any case, it was necessary to make use of the four-volume work now to be listed—

Jordan, David Starr, and Barton Warren Evermann. The Fishes of North and Middle America. Washington, 1896-1900. 4 vols. (vol. IV with 392 pls.).

Since all the synonymy is contained in the two latter works, it will be entirely omitted from consideration herein. References will be given to all the works consulted, but the general classification followed in this paper is that of Evermann and Marsh.

ON THE MORPHOLOGY, COLORATION AND BEHAVIOR OF SEVENTY TELEOSTS OF TORTUGAS, FLORIDA

I. Family Murænidæ. The Morays.

The moray eels are tropical and subtropical fishes lacking pectorals and having small round gill-openings and a thick leathery skin. They reach considerable size and are very voracious. Having their long narrow jaws filled with knife-like teeth and being decidedly pugnacious, they are very much dreaded. Not infrequently, when one is brought over the side of the boat, the fishermen vacate it. Three species were studied, one being new to Tortugas.

1. *Lycodontis vicinus* (Castelnau). Moray. Jordan & Evermann, I, 1896, p. 394.

Three specimens of this moray were taken in traps on July 5 and 7, 1914. So far as I know, it has never before been recorded from the Tortugas, and indeed it is new to the waters of the United States. However, there could be no doubt as to the classification. The teeth and, above all, the dark margin to the dorsal fin claimed it for *L. vicinus*. The lower jaw was provided with a number of long fixed canines in front, pointing backward; upper jaw with a number of the same kind fixed in the rim, and farther back a number of median ones set in membrane and all backwardly pointing and depressible. Behind these the vomer was beset with a single row of such teeth pointing toward the throat.

The wide mouth and throat opened into an equally wide œsophagus, showing some plications, and this with little change in diameter led into the long, straight, pouch-like stomach. Just at the point of junction of stomach and œsophagus, an aperture on the right side opened into a backwardly extending intestine. This intestine was underlain by the liver anteriorly, and posteriorly it was covered by what seemed to be the pancreas. It had in it nothing but mucus.

This fish breathed with its mouth open; its lower jawbones had a together-and-apart motion at the rear in a lateral horizontal plane; along its lower jaw and throat were curious folds in the skin which allowed for distension and contraction; inside the mouth, both in upper and lower jaws, were loose folds of tissue on each side, which approximated each other and receded during breathing, thus functioning as oral breathing valves. This membrane in the upper jaw was much thicker and more inflexible but, nevertheless, it functioned (as noted) in a rudimentary way. It was interesting to watch this moray breathing with its jaws quite one inch apart at the tip, with its lower jaw at the back widening out and narrowing, its throat ballooning and contracting, its opercular opening at one stage shaped like an O and at another collapsing and changing into a mere slit directed obliquely upward and backward. However, another specimen on one occasion breathed for a while very much like *L. moringa* (see below). Not only is the lower jaw distensible behind but the upper jaw also is so to some extent. Plate I, figure 1,[1] shows the changes in the width of the jaws and the functional breathing valves during respiration.

[1] This figure and the others to follow were drawn by Arthur Ohlman from the writer's rough sketches, the majority of them more or less semi-diagrammatic, made at Tortugas from the live fishes or from those that had just died.

2. *Lycodontis moringa* (Cuvier). Common Spotted Moray, Hamlet. Jordan & Evermann, I, 1896, p. 395, pl. LV, fig. 171; Evermann & Marsh, 1900, p. 77, fig. 8; Jordan & Thompson, 1905, p. 234.

Four specimens, all taken in traps, have been studied. Three only were measured, as follows: No. 1, length to tip of tail 2 feet 5 inches, of abdomen only 10 inches, of post-anal extension of abdomen 3 inches; No. 2, length 2 feet 3.5 inches, length of abdomen to anus 9 inches, post-anal extension 2.5 inches; No. 3, 3 feet 1 inch long, 3 pounds in weight. These morays agreed very closely with the descriptions and figures referred to above. The post-anal abdominal extension contained only the large urinary bladder and a large, club-shaped extension or lobe of the kidney.

It was very interesting to watch these morays breathe. They do not seem to have the loose folds in the mouth which serve as breathing valves in *L. funebris*, and they do not have the real oral valves so frequently found in other fishes. At each inspiration they closed the mouth and progressively contracted the muscles of its floor from before backward. Following this, the side walls and floor of the hinder part of the mouth assumed a curious bulbous or balloon-like shape and forced the water out at the small, round hole that does duty as an opercular opening. These morays swallow their breath very much as frogs do. Another was noticed to breathe with a curious gulping action accompanied by a widening out of the jaws behind.

Their feeding was likewise interesting. When small fish were thrown into the aquarium, a moray would seize one of these, catch as can, gradually work it around until the head pointed down-throat, and then by a series of convulsive throat movements and by aid of the backwardly pointing teeth would work it down. On one occasion there were two morays in a large aquarium at feeding time. One of these got the larger share of food, whereupon with every evidence of great anger the other seized this one by the snout and held it firmly. After a few minutes' writhings they broke apart, neither apparently the worse for the contest.

Two hamlets in one aquarium vomited their ration of fish, and the resulting fouling of the water, together with the stopping of the inflow, led them to climb out and die. Another did the same thing. One was distinctly seen to yawn one day, stretching its jaws and gaping as any other yawning animal would. The writer has noted yawning in about a half-dozen other fishes also.

3. *Lycodontis funebris* (Ranzani). Black Moray. Jordan & Evermann, I, 1896, p. 395; Evermann & Marsh, 1900, p. 77; Jordan & Thompson, 1905, p. 234.

The three specimens examined measured as follows: No. 1, length 3 feet 11 inches, girth 10 inches, weight 9 pounds; No. 2, length 4 feet 5 inches, weight 13 pounds; No. 3, length over all 5 feet 2 inches, to anus 2 feet 8 inches, of tail only 2 feet 6 inches, girth 1 foot 9 inches, depth 9.5 inches, weight 27 pounds. This last was the largest moray I have ever seen, and it was as active and savage as it was large. It was difficult to keep these morays in even fairly large aquaria, since during the night they would almost invariably climb out and die.

These fish agreed closely with Evermann & Marsh's and Jordan & Evermann's descriptions, differing, however, in two points: their jaws do completely close (these authorities must have had dead specimens with locked jaws), and in none of the three were there "dark lines running longitudinally" in dorsal and anal. There were faint lines present and these might

possibly become dark in the preserving fluid. Their general color was olive green. Nostrils appeared as paired tubes projecting forward. Three large sensory pores were noted on each jaw on each side, as shown in Plate I, figure 2.

The larger fish had in the middle front of the upper jaw a linear series of three backwardly hinging canine teeth, each 0.5 inch long. On the lower jaw was a similar series. In the smallest fish, the central tooth only was 0.5 inch long. Eye 3 in the snout, placed about midway between tip of snout and corner of mouth. The median-sized fish was a male whose paired testes, 1 foot long and 0.75 inch wide, were composed of flocculent tissue, like the fat body of a frog, reddish white in color.

The mouth in its structure and its actions is of particular interest. My notes for the fish 3 feet 11 inches long are especially full. Normally its mouth measured 1.5 inches straight across from angle to angle, but when distended its transverse diameter rose to 3.25 inches. This is due to the fact that the jaws, at the point where they are hinged and attached to the quadrate, are distensible in a horizontal lateral plane. In both jaws the large canine teeth, and in the upper jaw the teeth on the vomer especially, are hinged so as to be depressible backward. This allows food to be swallowed readily, but makes it almost impossible for anything to escape when once taken firmly between the jaws. The supra- and infrapharyngeals are longish bars, so set around the pharynx and so beset with backwardly hooked teeth as to greatly facilitate the swallowing process, as is shown in Plate I, figure 3.

In breathing the mouth is kept somewhat open and breathing valves appear to be present, but when a fresh fish was dissected no true permanent oral valves were found. Instead, however, loose folds of skin-like tissue above and below approximated each other in the median line and formed functional, but not structural, breathing valves. These disappeared entirely when the jaws and mouth were distended transversely. The ability to distend the jaws transversely in the rear to such a great width of course makes it impossible for oral valves to be present. The breathing is effected by the rising and falling of the floor of the mouth. The mouth and the gill cavities are lined with green skin.

The heart, which was placed very far forward, was a pear-shaped organ, the stem being the ventral aorta which gave off four branches on each side to the four gills. Each gill arch had in front of it a small round gill cleft. The heart was very active, continuing to beat for some time after death. The vascular system of all the morays dissected was well developed. Plate I, figure 4, shows the general form of the heart and the aorta with its branches.

Of no less interesting structure were the parts of the digestive apparatus, shown in Plate I, figure 5. The wide œsophagus underwent a slight constriction at the point where it joined the stomach. This latter organ extended straight backward almost the full length of the abdomen, diminishing into a small finger-shaped appendix at its hinder end. This pouch-like organ was much plicated internally, save in the small posterior part which was entirely free from such folds. Thus œsophagus and stomach are continuous, but, at the point where the stomach proper begins to widen out back of the œsophageal constriction, there was given off either on the dorsal side or slightly to the right a long slender intestine which ran straight back to the anus. This was divided internally into an immense number of

crypts, and externally for a large part of its anterior length was covered with liver and pancreas. The major portion of the liver, *i. e.*, that part adjacent to the gall-bladder, together with this organ, lay immediately ventrad to the junction of stomach and gut, was somewhat displaced to the right, and was grown fast to the wall of the abdomen. Thus, looked at dorsally, the organs are in the following order: gut, stomach, liver. One moray had in the stomach a much decomposed fish, head down; another, some teleostean vertebræ; and the third, while in the aquarium became sick and vomited four 6-inch grunts.

In the 5-foot-2-inch specimen the length of the abdomen proper measured 2 feet, but there was a post-anal extension of 6 inches containing the large swollen end of the kidney and the urinary bladder. An air-bladder was present.

II. Family Clupeidæ. The Herrings.

Of this large family, probably several genera are found in the Tortugas, but only one was examined. Fishes of this family are given to swimming in great schools at or near the surface of the sea, where, by means of their close-set gill-rakers, they sieve out of the water the plankton which serves them for food.

4. *Jenkinsia stolifera* (Jordan & Gilbert). Jordan & Evermann, I, 1896, p. 419; Evermann & Marsh, 1900, p. 84; Jordan & Thompson, 1905, p. 232.

These little fishes abound along the shores of the keys. Four were measured: 1.5, 1.8, 1.88 and 1.94 inches over all. These specimens conformed very closely to Evermann & Marsh's description. On the head, between and back of the hinder edge of the eyes, was a fair-sized spot shaped like a conventional drawing of a heart but having a rounded, instead of pointed, base, as shown in Plate I, figure 6. Around the bottom of the U-shaped inpushing was a dense agglomeration of black dots; elsewhere these were scattering. The whole area of the heart-shaped spot was underlain with a beautiful blue, intermediate between a royal purple and a hyacinth blue. This was most marked in the lateral region of the horns of the U. The top of the eye showed the same color.

These little fishes were found in great schools, numbering probably millions. They were especially abundant on the lee side of Loggerhead Key and around our wharf. They often hover over gray snappers, which seem to feed on them. However, the snappers either excite little fear in the herrings or else possess a great fascination for them. The little fish collect around them in dense swarms, resembling a swarm of bees, leaving a clear space immediately around the snapper about equal to his length, as shown in Plate I, figure 7. Whenever the snapper moves, the rather orderly swarm breaks up into a mob, which quickly rearranges itself when the big fish comes to rest. The little fish are in motion all the time, but the swarm remains fairly constant in size and shape.

III. Family Esocidæ. The Needle Fishes.

Fishes with long, slender, compressed bodies; with jaws produced into a slender snout and armed with sharp close-set teeth. These fishes are much given to leaping, especially when startled, and at night are attracted by lights. Dr. Mayor told me that he had known men fishing on the reef by torchlight to be seriously hurt by them.

5. *Tylosurus marinus* (Walbaum). Gar Fish, Bill Fish, Needle Fish, Green Gar. Jordan & Evermann, I, 1898, p. 714.

One small specimen was taken, but no measurements were made. It was noted that, when the mouth was opened, not only was the lower jaw depressed but the upper was raised. Eye with pupil shaped as in the diagrammatic figure, Plate I, figure 8. No dark bar on front of opercle. The lateral line, which ran along the sides of the belly, did not end in the median lateral keel on the caudal peduncle, but slightly under this. This fish swims at the surface in shallow water, among shoals of small fishes, on which it feeds. It has not heretofore been reported from the Tortugas.

This fish seems to be a favorite food for the jacks. On June 9, 1913, I saw a school of jacks snapping at a small group of green gars off the southern coal-shed wharf at Fort Jefferson. Every one of the gars had lost its caudal fin, and all were swimming feebly with their stumps. The jacks seemed to snap mainly at the caudal parts of the gars and were making the water fairly boil.

6. *Tylosurus raphidoma* (Ranzani). Hound Fish. Jordan & Evermann, I, 1896, pp. 715-716, pl. cxvi, fig. 308; Evermann & Marsh, 1900, pp. 99-100, fig. 17; Jordan & Thompson, 1905, p. 234.

Two specimens were studied: one killed by Dr. S. C. Ball, with a shotgun off the western dock, Loggerhead Key; the other caught in a gill-net off the eastern dock. The latter was carefully measured, with the following records: extreme length 2 feet 6 inches, to base of caudal 2 feet 2.5 inches, to anus 1 foot 8.5 inches, of head 8.25 inches, of jaws 4.75 inches; depth 2 inches, girth 6, spread of caudal 4, width across back between pectorals 1.88, diameter of eye 0.75 inches.

The fin-ray count for this fish was D. I, 20; A. I, 20. Evermann & Marsh, who copy Jordan & Evermann in both key and text, say in their key D. I, 21-24; A. I, 22-24; and in their text D. 21-24; A. 22-24. The figure (identical) used in both works shows D. I, 21; A. I, 19.

The eye was much larger and the jaws much longer in proportion to the rest of the head and body than in the figure referred to. In both jaws the big vertical canine teeth were set slightly inside the irregular rows of small, needle-like teeth. At the angle of the jaws these canines became shorter and heavier, more bluntly conical with relatively larger bases, and pointed backward. The lips of both jaws were roughened by a multitude of horny tubercles, which extended outward in an area nearly one-eighth of an inch wide in the largest specimen. The lower jaw was wider than the upper one and when the mouth was closed the lower canines lay outside the upper jaw and along its tubercle-roughened lip. In the lower jaw the floor of the mouth (seemingly a continuation of the tongue) was separated from the jaws by a narrow fissure on each side. Into this fissure the canines of the upper jaw fitted when the mouth was closed. Thus a sardine or other small fish is securely held when pierced by these saber-shaped teeth. The snout was quite pointed but not "unusually short, stiff, strong, rapidly tapering forward," and a large fish making a vigorous leap (as they are prone to do) might well be dangerous to a fisherman, especially if naked. The mouth contained a pair of oral breathing valves, one above and one below. Externally the floor of the mouth was composed of loose material, soft and movable entirely to the symphysis of the lower jaw.

The pelvic fins were inserted exactly midway between middle of eye and base of caudal, as stated in both texts above cited and as shown in the figure. The dorsal extended backward beyond the anal by 3 rays, exactly as the figure shows. The lower lobe of the caudal was notably longer than the upper and was much darker—the tip, however, being transparent. The lateral line ran low on the belly and ended behind in the median lateral keel.

This fish was a female with spent ovaries, the right lobe was 6.5 and the left 4 inches long. The liver was compressed into 3 long narrow lobes; the median one shortest, the left longer, the right one longest (5 inches) with the gall-bladder imbedded in its outer margin, as shown in Plate I, figure 9.

The hound fish, like the other gars, loves to lie motionless or to swim idly to and fro amid schools of small fish, around wharves, piles, wrecks, etc., where it often serves as prey for the barracuda. In 1912 considerable numbers hung around our western dock at Loggerhead, and I frequently tried to kill them with a small-caliber rifle. I disabled a great many but never got one, for they were at once snapped up by hungry barracudas which circled around just out of gunshot. Jordan & Thompson note the fondness of barracudas for this same gar.

Found along the shores at times when considerable quantities of broken bits of eel-grass abound (which they markedly resemble), are certain small, protectively colored fish which Dr. Mayor called to my attention under the appellation, stick-fish. Numbers of these were taken, and were found to have paired fleshy lappets at the extremity of the elongated, lower jaw. These little fish have been identified as the post-larval young of *Tylosurus raphidoma*. A colored drawing was made and careful notes recorded. At the American Museum I have examined older specimens showing the gradual disappearance of these lappets. This information will be brought out in a special paper.

IV. Family Mugilidæ. The Mullets.

The members of this numerous and widely diffused family live in fresh waters or are found as marine forms along the shores in warm regions. Characteristic are the hard dorsal, always with 4 spines, the small mouth, and (since they feed on the organic matter contained in the mud along the shores) a long intestinal tract to provide the necessary digestive and absorptive surface. Jordan & Thompson report three species from Tortugas but I have had only one.

7. *Mugil curema* Cuvier & Valenciennes. White Mullet. Jordan & Evermann, I, 1896, p. 813, pl. cxxvi, fig. 344; Evermann & Marsh, 1900, p. 113, fig. 24; Jordan & Thompson, 1905, p. 235.

The one small specimen studied was 2.25 inches over all. Mouth somewhat protractile. Teeth hair-like. Lower jaw acutely pointed at the tip. Inside of gill-covers dotted with scattering small round black dots becoming somewhat denser on upper hinder region. Pseudobranchiæ very pronounced. Scales on dorsal and anal fins of this small specimen visible only under compound microscope. Pelvics with median accessory scale. No lateral line but centers of series of scales so marked that the scales line up in such a way as to give the appearance of multi-lateral lines, as shown in Evermann & Marsh's excellent figure referred to above.

Alimentary canal composed of a muscular gizzard-like stomach and a much and curiously twisted gut. The saddle-shaped liver lay ventrad to the anterior end of the digestive tract with the gall-bladder between it and the stomach-gut combination. Gall-bladder blue-green in color but covered with the black peritoneum which lines the abdomen.

The specimen described came from a tide pool on the east side of Loggerhead Key. It is found abundantly everywhere along shores.

V. Family Sphyrænidæ. The Barracudas.

Active, voracious, carnivorous fishes of warm oceans; the marine counterparts of the fresh-water pikes and muskellunges. The large forms, having large jaws fitted with knife-blade shaped fangs, are fierce and fearless and much more dangerous, generally speaking, than the ordinary sharks. The only species collected by me at Tortugas is the large form.

8. *Sphyræna barracuda* (Walbaum). Barracuda, Picuda. Jordan & Evermann, 1898, p. 2841, pl. CXXVIII, fig. 349; Evermann & Marsh, 1900, p. 115, fig. 26; Jordan, 1904, p. 539; Jordan & Thompson, 1905, p 236.

On this fish I have published an extensive paper entitled *Sphyræna barracuda; its Morphology, Habits and History* (Papers from Department of Marine Biology, vol. 11, Carnegie Institution, Washington, Pub. 252, 1918, pp. 53-108, 5 text-figs. and 7 plates). To this paper the interested reader is referred.

One section of this paper was devoted to a discussion of the question of its attacking man, and it was shown that generally speaking it is more dangerous to man than the ordinary shark. Later, in collaboration with C. M. Breder Jr., a specific attack on a man at Colon has been carefully described and illustrated with photographs of the wounds. All the available data for the ferocity of this fish has been brought together in this article.[1]

VI. Family Holocentridæ. The Squirrel Fishes.

The interesting family of squirrel fishes is known in the Tortugas by three species, on all of which careful notes have been made. The first, *Holocentrus ascensionis*, has long been known, but was never taken by Dr. Thompson. The second, *H. siccifer*, was known only from the Bahamas until taken at the Tortugas by Dr. Thompson. While to the same enterprising collector we owe the third and new species, *H. tortugæ*. All of these are beautifully colored fishes found around coral reefs in water 20 or 25 feet deep.

9. *Holocentrus ascensionis* (Osbeck). Squirrel Fish. Jordan & Evermann, I, 1896, pp. 848-849, pl. CXXXI, fig. 358; Evermann & Marsh, 1900, pp. 118-119, pl. 3; Jordan & Thompson, 1905, p. 236.

Two specimens were taken with dynamite on Loggerhead Reef. Their measurements were as follows for each respectively: length over all, 7.5 and 9.5 inches; to base of caudal fin, 5.9 and 7.5 inches; depth, 2 and 2.5; length of head, 2 and 2.5; diameter of eye, 0.6 and 0.8 inches. Each had fin rays: D. XI, 15; A. IV, 10; pelvics, I. 7. Colors essentially as given by Evermann & Marsh and shown in their elegant figure. Tip of soft dorsal and of pelvic fins red, as shown in figure but not in description. Pelvics

[1] E. W. Gudger and C. M. Breder Jr. The Barracuda *(Sphyræna)* Dangerous to Man. Journal American Medical Association, 1928, vol. 90, pp. 1938-1943, 5 figs.

very long and pointed, reaching to or past the anus. Caudal markedly forked, the upper lobe being much the longer and more pointed. Caudal peduncle very small and slender, its width about half its depth. Caudal with 5 spines above and 4 below. All scales spiny. Premaxillary spine and its groove in forehead very marked, being covered only by a thin skin. Head parts beautifully mailed and sculptured, spiny everywhere, especially on the opercular parts. I know of no fishes so mailed and spined save the scorpænas and the sea-robins of the genus *Prionotus*. Mouth very protractile and much enlarged when so protruded, evidently intended to take in food of good size.

10. *Holocentrus siccifer* Cope. Jordan & Evermann, I, 1896, pp. 849-850; Jordan & Thompson, 1905, p. 236.

Five specimens were taken, one alive from a tide-pool on the east side of Loggerhead Key, and four with dynamite from Loggerhead Reef on the west side of the Key. Four of these had fin rays normal as given by Jordan & Evermann; D. XI, 13; A. IV, 9; one, however, had 14 dorsal spines instead of 13. Three of these fishes were measured, two in inches and one in millimeters for the sake of even greater exactness. The two measured in inches show some interesting variations in size: length over all 6.25, 6.25; to base of caudal 5.25, 5.37; depth 1.5, 1.75; length of head 1.6, 1.8; diameter of eye 0.6 and 0.6 inches. The other specimens were all small, the longest measured in length between perpendiculars 92 mm.; to base of caudal 76 mm.; depth 29; length of head with spines 27; diameter of eye 11; interorbital width 8.5, length of snout 6 mm. These last measurements give an accurate basis for comparative measurements to contrast with Jordan & Evermann's (in parentheses). Head with spines in body 2.8 (2.75); depth in body 2.6 (2.5); eye in head 2.5 (2.6); eye in interorbital width 0.77 (0.75)—a very close approximation. My scale count was 3-44-8; theirs 3-45-8.

Caudal fin (two specimens) with 6 spines above and 5 below. Opercular region much spined all over. Preopercle with a very large spine at angle. On upper edge of opercle 3 spines, 2 equal and of fair size. At angle of opercle 2 large, twin spines having a single base, as seen in Plate I, figure 10. Opercle covered with 9 scales growing smaller downward, all very spinous. Nape covered with large multi-spinous scales. All scales everywhere spinous.

Life-color (two specimens) was as follows: Reddish-brown with longitudinal silver stripes, or, perhaps better, silvery with longitudinal reddish-brown stripes; a median broad stripe of reddish (having a lighter central area) running from eye into caudal peduncle; another dark stripe just under the dorsal fin; between this and the mid-lateral stripe was a third dark bar having the lateral line running in its center. This stripe coalesced with the mid-lateral one on the caudal peduncle. In all there were about 10 dark stripes on the side separated by lighter ones. Dorsal fin with black patch between spines 2 and 3 (as noted by Jordan & Thompson), darkish-red between spines 3 and 4; red with less dark between 4 and 5; back of 5 a red bar across the top of the spines and a vertical red stripe covering the half of the membrane anterior to each spine, the half of the membrane posterior to the spine colorless—the colorless and red parts of the membrane between any two spines being about equal, all as shown in Plate I, figure 11. First soft dorsal ray red, others yellowish. The two outer caudal rays above and below red, balance of fin yellow. Third anal spine and first soft ray scarlet red, balance of fin yellow. Pelvics with a median much-

toothed accessory scale, first soft ray decidedly red, remainder of fin yellowish. Pectorals pink with black axillæ.

As Jordan & Thompson remark, this species is very near to *H. vexillarius*, and despite the difference in scale count I am inclined to think it is identical with *H. vexillarius*. The band, the color of the fins, the black axil of the pectoral and the spines on forehead and opercle are almost identical with those for this latter species. To have live specimens of the two species seems to be the only way to settle this vexed identification.

11. *Holocentrus tortugæ* Jordan & Thompson. Jordan & Thompson, 1905, p. 236, fig. 1.

One specimen was taken with dynamite on Loggerhead Reef. It measured: length over all 116 mm.; to base of caudal 97 mm.; depth 27 mm.; length of head 33 mm.; diameter of eye 12 mm. Jordan & Thompson's figure and description were both made from a preserved specimen. My notes were made on this specimen killed instantly and examined when just out of the water.

There were 5 caudal spines above and 4 below. Accessory scales were present between the pelvic fins. The opercular spines corresponded exactly with those shown in Jordan & Thompson's figure. When the mouth was closed, the fitting together of the upper and lower lips, when looked at from above, gave an exact representation of a mule shoe, the hinder diameter or width being narrower than any other. The reverse was the case, however, when the mouth was opened, the width from corner to corner being greater than any other width. This was due to the fact that, when protruded and distended, the maxillæ spring widely apart behind. These changes are shown in Plate I, figure 12, A and B.

Body silvery, with 9 red stripes on the sides between 10 silvery ones. On the nape a median red stripe ran to the anterior base of the dorsal fin. Converging with this red stripe at this point were two lateral red stripes, running from the head. These united made a dark red base for the spinous dorsal and reappeared behind the soft dorsal as a median red stripe on the caudal peduncle. The dorsal spines were set heteracanthus (staggered) in their groove. The black patch on the spinous dorsal, shown in Jordan & Thompson's figure as covering 3 spines and 3 membranes, in my specimen covered 3 spines and only 2.5 membranes. Beginning with membrane 3, at the top behind the tip of the spine there was a transparent triangular region, below this a broad red band extended across the membrane, below this a transparent band about three-fourths as wide. This arrangement of shaded (red) and colorless regions was continued throughout the whole spinous dorsal, becoming narrower and descending lower as the fin rays became shorter. For a representation see Plate I, figure 13.

With the three Holocentri before one, it is very clear that we have 3 distinct species. *H. ascensionis* is clear red with a slender caudal peduncle; *tortugæ* is also clear red but with a short heavy peduncle (heavier than in Jordan & Thompson's figure), and *siccifer* is a dark heavy short fish. It may be added here that, when worked out, the number of caudal spines, which seems fixed and distinctive, may be found of diagnostic value.

VII. Family Scombridæ. The Mackerels.

The mackerels are readily distinguishable by their fusiform bodies, by the continuation of the dorsal and anal fins backward in a series of finlets,

and by their wide-forked tails. Built for speed, they show it in their beautiful "stream line" modeling. Three species were collected at or near Tortugas, one of these being new to the Tortugas fauna.

12. *Gymnosarda alleterata* (Rafinesque). Little Tunny. Jordan & Evermann, I, 1896, p. 869, pl. CXXXIV, fig. 366.

The only specimen of this interesting fish recorded from the Tortugas was caught by the writer while trolling between Loggerhead and Bird Keys on June 5, 1913. Careful measurements give for the length between perpendiculars, 2 feet 3 inches; to notch of caudal, 2 feet 1.5 inches; to base of caudal, 1 foot 11.5 inches; of head, 6.5 inches. Other measurements are: depth, 5.25 inches; greatest girth, 1 foot 3 inches; smallest girth, at "small" of tail, 2.5 inches; diameter of eye, 0.9 inch; height of first dorsal spine 3.5 inches; spread of caudal, 7.25 inches.

The fin-ray count of this specimen, D. XV-I, 12-VIII, A. III, 11-VII, does not agree with Jordan & Evermann's, D. XV-I, 12-VIII, A. 12-VII. The lateral line was without any marked bend. Tip of pectorals extended back under eleventh dorsal spine (not the ninth). The spinous dorsal was depressible so as to be completely out of sight in its groove, hinder part normally so; all at will. Pectorals and pelvics likewise had depressions in the corselet (described in the next paragraph) in which, when in motion, they fitted accurately, thus giving the body a perfectly smooth surface anteriorly—*i. e.*, this fish is built for speed.

The minute cycloid scales of this fish form an interesting armor-like structure called a corselet, which covers the neck and shoulder region. On the mid-dorsal region this sends back a narrow peninsula-like extension, in the center of which the spinous dorsal arises. Just below this on each side is a narrow bay projecting forward in the corselet and devoid of scales. Below this is a backward extension of the corselet bearing the lateral line. Under the pectoral is another bay in the corselet. Between pectoral and pelvic the corselet sends another projection backward on each side. The pelvics are set in another bay in the corselet. In other words, pectoral and pelvic fins have bays in the corselet in which they fit accurately and smoothly when at rest. With the dorsal depressed and pectorals and pelvics out of the way in their depressions, the surface of the fish is perfectly smooth, offering no resistance to motion.

Color about as in Jordan & Evermann. Above the lateral line could be counted about 17 oblique wavy blue streaks. Below the left pectoral there were 6 round dark blue (not black) spots about the size of the eye, below the right pectoral 5 were arranged wholly differently from the others.

The internal organs of this fish are not less interesting than the external structures. The paired ovaries, containing unripe eggs, were 8 inches long and extended forward almost the full length of the abdomen. The liver was, comparatively speaking, enormous in size, amounting to from 40 to 50 per cent of the volume of all the viscera. In position it was ventrad to all the other organs. The gall-bladder was very long and slender—3 inches long by 0.25 to 0.4 inch wide. The digestive tract was unusual in structure. The œsophagus extended backward into a narrow pouch 7.5 inches long, slightly enlarged in the middle section. The walls of this were very thick and rugose, much more so than those of the stomach of a frog. At the anterior end of this pouch, just below the œsophagus, a small thick-walled tube was given off on the right side. This small tube was soon constricted (the pyloric valve ?), and beyond the constriction the canal

widened out into a pouch-like organ having thin walls slightly plicated. This section of the canal was twisted twice (somewhat in the shape of an S) and lay imbedded in the substance of the liver. At the point where the canal widened into the sac-like enlargement there were given off and extended into the substance of the liver a number of diverticula, the pyloric cœca common to the Scombridæ. These opened by wide mouths into the thin-walled part of the alimentary canal just back of the pyloric constriction. These structures (omitting the diverticula) are to be seen in Plate II, figure 1.

The air-bladder was wanting. The heart was separated from the abdomen by the pericardial wall which formed a so-called diaphragm. The heart was almost in the form of a solid isosceles triangle with the bulbus aortæ at its apex.

13. *Scomberomorus regalis* (Bloch). Jordan & Evermann, I, 1896, p. 875, pl. cxxxv, fig. 369; Evermann & Marsh, 1900, p. 124, fig. 28.

Three specimens of this fish have been studied. They measured as follows: No. 1, length to tip of caudal 2 feet 6.75 inches, to base of same 2 feet 1 inch, girth 11 inches; No. 2, length to tip of caudal 2 feet 4 inches, to fork of same 2 feet 1.25 inches, to base 1 foot 11.5 inches, to front edge of anal 1 foot 2.75 inches, to front edge of dorsal 1 foot 1.75 inches, of head 6 inches, diameter of eye 0.75 inch, girth 11 inches; No. 3, outside length 2 feet 6.5 inches, to base of tail fin 2 feet 1.5 inches, girth 1 foot, spread of caudal 8 inches, weight 6 pounds.

There seems to be much variation in the fin rays of these fish, and the data will be given in full. No. 1 had D. XVI-17-VIII, anal not counted. No. 2 had D. XVI-17-VIII, A. II-14-IX. No. 3 had D. XVI-17-VIII, A. III-14-VIII, and in this specimen spine III did not reach to the tips of the soft rays. Evermann & Marsh say D. XV-I-15-VIII; A. II-15-VIII. Jordan & Evermann give D. XVII-I-15-VIII; A. II-14-VIII. Their figures, which are made from the same block, have D. XVII-13-VIII; A. —,[1] 15-VIII. This is not a fair count of the soft dorsal rays, since in this small figure they are not so drawn as to be clearly visible. The same is probably true of the anal spines. Further, in the figure it is hard to decide whether the last part of the soft dorsal is a part of that fin or is to be counted as a finlet. Now this is drawn correctly, for it was expressly noted of fish No. 2 that the last ray of the soft dorsal was shaped like a finlet but that it moved when the soft dorsal was moved. It is evidently in the transition stage from the soft dorsal into the finlets. It may be noted here that the soft dorsal is inserted slightly—about the diameter of the eye—in front of the insertion of the anal.

In color these specimens agree very well with the description of Evermann & Marsh (Jordan & Evermann's figure is quite defective, *e. g.*, in the two longitudinal bands). Both sets of authorities had preserved specimens only, as is witnessed by "black longitudinal band." The above three specimens in life had a bright orange-yellow band beginning above the base of the pectoral, running backward on to the caudal peduncle to a point under the last finlet. The lateral line began above the origin of the yellow bar, crossed this under the anterior base of the soft dorsal, and ended in the median lateral keel on the caudal peduncle. Above the yellow bar, No. 1 had one row of orange spots, below it 5 rows of oblong spots not all con-

[1] The anal spines can not be made out.

tinuous from head to tail, most marked between pectoral and beginning of soft dorsal. No. 2 had 2 rows of spots above the bar and 4 below. The spots on No. 3 were not noted. In all three fish the spinous dorsal was black in front and above, and white below—essentially as in the figure. This mackerel has along the middle of each side a large keel, and above and below this smaller keels—3 on each side of the fish.

This large mackerel is a deep-sea fish and is taken only in blue water. Mine were taken trolling behind the *Dohrn* as she moved along at 7 or 8 miles per hour. No. 1 near Rebecca Light, No. 2 off Woman Key, No. 3 near the sea buoy of Southeast Channel at Tortugas. They were taken on the same trip in rough weather, which made it impossible for me to dissect them carefully.

This scombrid, whose specific name is very properly *regalis*, since it is a royal fish in appearance, in gameness and when served on the table, sometimes grows to a length of 6 feet and a weight of 25 pounds. So far as known to the writer, it has not heretofore been reported from the Tortugas.

14. *Scomberomorus cavalla* (Cuvier & Valenciennes). King Fish, Cero. Jordan & Evermann, I, 1896, pp. 875-876; Evermann & Marsh, 1900, pp. 124-125; Jordan & Thompson, 1905, p. 237.

The one specimen taken, measured: length over all 2 feet 2 inches; to fork of caudal 1 foot 10.5 inches; to base of same 1 foot 8.5 inches; of head 5 inches; spread of caudal 7.25 inches. Dorsal fin with 16 spines. This fish was a female with ovaries of considerable size. It was taken on a trolling line behind the *Dohrn* about 2 miles west of Rebecca Light. The weather being stormy, it was impossible to dissect it, and beyond identifying the fish, no notes were made.

This is the largest of the Spanish mackerels, sometimes reaching a weight of 100 pounds, while 40 pounds is not unusual. However, the usual weight in the Florida Keys is about 10 pounds. The fish go in schools and are readily caught by trolling. The flesh is firm and of fine flavor, and the fish are much sought for the market.

VIII. Family Carangidæ. The Pompanos.

The outstanding features of these fishes, which catch the eye at first glance, are the greatly elongated dorsal and anal fins; the generally elongated falcate pectorals; the usually strongly developed scutes on the sides of the caudal peduncle; and finally, the deeply forked caudal. Most of these fishes are rapid swimmers and voracious feeders. Three forms have been studied at Tortugas.

15. *Caranx ruber* (Bloch). Crevalle. Jordan & Evermann, I, 1896, p. 919; Evermann & Marsh, 1900, pp. 130-131.

My one specimen was taken in a seine on the east side of Loggerhead Key. Its fin formulæ were D. VIII-I, 27; A. II-I, 24. Evermann & Marsh give D. VIII-I, 26; A. II-I, 23; and Jordan & Evermann D. VII-I, 26; A. II-I, 22. The scutes were about 25. Members of the family Carangidæ are much given to variation and are notoriously hard to classify.

The falcate pectoral fins were very long, longer than the head, reaching back to the eighth or tenth ray in the soft dorsal, to the dip in it, and almost to the point where the lateral line began to bend down. This soft

dorsal was found to be partly depressible. The lower lobe of the caudal was very dark.

This species seems to be uncommon at Tortugas; at any rate, it has not been heretofore recorded.

16. *Caranx bartholomæi* Cuvier & Valenciennes. Yellow Jack. Jordan & Evermann, I, 1896, pp. 919-920; Evermann & Marsh, 1900, p. 131; Jordan & Thompson, 1905, p. 237.

Of this fish also only one specimen was taken, this in a trap at the wharf at Loggerhead Key. Its fin count was D. VIII-I, 27; A. II-I, 23. In front of the spinous dorsal were four hard chitinous humps or ridges looking like remnants of dorsal spines. The spinous parts of both dorsal and anal were depressible in sheaths which were flush with the back, *i. e.*, did not stand up. The soft parts of both fins have upstanding scaly sheaths, that of the former being 0.25 to 0.375 of an inch high. The caudal peduncle was strongly keeled on each side, the serrated scutes of the lateral line being so prominent as to make the peduncle decidedly flat above and below. These ended abruptly at the origin of the caudal, giving the tail part of the body a very stumpy appearance, as shown in Plate II, figure 2. The pectorals were markedly falcate, and, reaching beyond the origin of the soft anal, they extended exactly to the point where the bend in the lateral line occurred. The caudal was deeply and equally forked, as shown in Plate II, figure 3, a tracing from the tail itself. The body was elliptical in cross-section and the forehead had a beautifully regular curve.

In color the fish was bluish, *e. g.*, a silver over purple. On either side of the dorsal was a bluish-green band, below this a purple stripe beginning on the head just over the eye and extending to the hinder base of the soft dorsal. This widened out considerably over the base of the pectoral. Corresponding to this, there was on either side of the base of the anal a similar stripe, silvery in color, while just above this, on either side, a parallel purple stripe extended backward. These purple stripes originated on the median line just in front of the anus.

There was some doubt as to the identification of this fish, but it agreed most closely with the diagnosis of the authors quoted for *C. bartholomæi*, and it could not be any other member of the family. It seems to be rather rare at the Tortugas, the only other specimen recorded being one "seen among coral heads, southwest shoal," as noted by Thompson (Jordan & Thompson, 1905).

17. *Trachinotus glaucus* (Bloch). Gaff-topsail Pompano. Jordan & Evermann, I, 1896, pp. 940-941, pl. CXLVI, fig. 395; Evermann & Marsh, 1900, pp. 137-138, fig. 37.

My one specimen measured: length over all 4.88 inches, to base of caudal 3.88; depth 1.75 inches. Fin-ray formula D. I-VI-I, 19; A. II-I, 17. Evermann & Marsh agree with Jordan & Evermann (whom they have simply copied) on D. VI-I, 18; A. II-I, 18. Their figure, which is common to both works, has D. VI-I, 20; A. II-I, 17. Neither pair of authors takes any notice of the procumbent spine in giving this dorsal formula, yet it is present, and in my specimen, while shorter than any of the others, was likewise stouter. It was situated forward of the other spines, lay under the skin, and pointed forward. These authorities do take notice of this elsewhere, for, when they came to locate the vertical stripes, they put the first one "under the procumbent spine"—where it is found.

All dorsal spines (except the procumbent one) were depressible backward in a sheath. Soft dorsal and anal, both lobes of caudal, and upper part of pectorals dark in color. Lower part of pectorals almost colorless. Caudal of this specimen with upper lobe bluntly rounded as shown in Plate II, figure 4, probably the result of a bite by an enemy. Dorsal and anal fins not reaching quite to base of caudal, not long and pointed as shown in the figure referred to above, possibly the result of age or accident. Four vertical bars as shown in their figure, first under procumbent spine; second just behind spine 5; third under spine 6, much farther forward than in their figure. Fourth bar very faint, and no trace of fifth in the form of a dark spot on caudal peduncle. Likewise no marks on preopercle as shown in their figure.

The abdomen had a slight paired post-anal extension, on either side of the roots of the anal spines, filled with the short horns of the air-bladder. There were 14 pyloric cæca instead of the 13 called for. The large gall-bladder, which with the liver lay dorsal to the coiled intestine, was four times longer than wide, club-shaped and covered with the bluish-green iridescent peritoneum.

This fish, which may reach a length of a foot, is called gaff-topsail pompano, because of its long, pointed, soft dorsal fin which the soft anal approaches in length. These fins are generally carried high, giving the fish a striking appearance. The generic name means rough back, in reference to the short stout spines. The term *glaucus* means hoary blue in allusion to the general color of the fish. This fish is not particularly esteemed for food.

IX. Family Pempheridæ. The Deep-water Catalufas.

The Catalufas are small tropical fishes inhabiting fairly deep water. They are not very well known and their affinities are rather doubtful. Three specimens of one species only were taken.

18. *Pempheris schomburgki* Müller & Troschel. Jordan & Evermann, I, 1896, p. 978.

Three specimens were taken off west side of Loggerhead Key with dynamite. The fin-ray count was D. IV, 9; A. III, 33. The measurements of the medium-sized fish were as follows, in millimeters: length to top of caudal 60, to base of caudal 48, of head 17; depth 20; diameter of eye 8; of interorbital space 5; length of base of anal 23, of longest ray of anal 7 mm. The comparative measurements do not at all agree with the authorities. They say head 4 (mine, 2.8), depth 3 (2.4), eye 3.5 in head (2.1), anal base in body not quite 2.5 (2.1), its first rays 3.5 in head (3.3)—the measurements in parentheses being those of my specimen.

Color of fresh specimen, coppery bronze above, darker behind, silvery on belly. Olive green on head and around mouth, dark green along base of dorsal. Anterior part of dorsal greenish yellow with dark specks. Caudal reddish with dark specks, lunate with upper lobe longer and darker. Anal fin very long, the scales at its base dark, almost black. Pectorals and pelvics pinkish—due apparently to blood-vessels along the rays. Pelvics apparently with median accessory scales, though these may be merely a number of ordinary scales. Eye very large (2 in head) and prominent. Lateral line very marked, extending through caudal.

The largest specimen, which was merely stunned, was kept in an aquarium for some days. Its mode of progression was very marked. It swam by means of its pectorals only and moved with a curious jumpy motion like the

hopping of a sparrow or perhaps (though less) like the galloping flight of that member of the woodpecker family known as the Yellowhammer. When swimming, the caudal moved to and fro but was not used as an agent of propulsion.

Jordan & Thompson list *Pempheris mülleri* from the Tortugas. Their description, however, agrees more closely with that of *P. schomburgki*, and Jordan & Evermann say (p. 979) of this 5-inch specimen that it is "probably identical with *P. schomburgki.*" If Jordan & Thompson's identification stands, this is the first record of *P. schomburgki* for the Tortugas, and indeed for the United States, so far as I know.

X. Family Cheilodipteridæ. The Cardinal Fishes.

The members of this large family of tropical fishes are noted for the sharply separated spinous and soft parts of their dorsal fins, and for their rather large scales. Their color is frequently a bright red, and many species carry their eggs in their mouths until they are hatched. All our laboratory men, Florida and Bahama born, declared that they had never heard of such a habit in these fishes. I have had only three species, but since I left Tortugas others have been taken, some practicing oral gestation. These fishes are found around and under coral heads and are best taken by the use of dynamite.

19. *Apogon pigmentarius* (Poey). Jordan & Evermann, I, 1896, p. 1109, pl. CLXXVIII, fig. 472.

Only one specimen of this interesting little fish was taken. Its length over all was 3 inches; to the base of the caudal 2.25 inches; depth 0.75 inch. This specimen differed considerably from Jordan & Evermann's description. The head was 3 instead of 2.5 times in the length, and the eye was contained 2.8 times in the head instead of 2.25. The dorsal fin formula was VI-I, 9, while theirs was VI-I, 8; and the lateral line scales were 2-25-7, contrasted with their 3-25-3. But for all these differences, the two fish were the same, the scales were everywhere "fly-specked"—*pigmentarius*, dotted—and in other points this specimen agreed closely with Jordan & Evermann's description and figure.

The maxillary reached just to the hinder edge of the pupil. The gill-rakers on the front side of the first arch were very long and slender (three times as long as any of the others) and pointed toward the mouth. The general color of the fish was a carmine red. The spinous dorsal was fairly dark, the soft part pink. The caudal was pink edged with dark; all the other fins were a rose-pink. The gill-covers and sides of the head were purplish.

This fish, which has not been heretofore reported from the Tortugas, or from the United States, was first described and named by Poey, the distinguished Cuban ichthyologist, who found it common at Havana.

20. *Apogon sellicauda* Evermann & Marsh. Evermann & Marsh, 1900, p. 143, fig. 40; Jordan, 1904, p. 539; Jordan & Thompson, 1905, p. 239.

Seven specimens of this, the most striking fish studied at Tortugas, were examined. These ranged from 28 to 90 mm. long over all, and all were so characteristically marked that there was no trouble whatever in classifying them. The largest measured: length over all 90 mm.; to base of caudal 77 mm.; depth 25 mm.; length of head 27 mm.; of snout 6 mm.; diameter

of eye 10 mm. This gives us for proportional measurement: head 2.9 (Evermann & Marsh 2.6); depth 3.1 (3); snout 4.5 (4.75). The dorsal fin formula was VI-I, 9; the anal II, 8 (having 1 more soft ray than the Porto Rico specimen.) The scales were 2-29-9. My other specimens agreed with that from Porto Rico in fin-ray formulas, and their lateral lines had 28 and 29 scales.

The living fish was very striking in appearance, being a vivid scarlet in color and having on the caudal peduncle a darker red saddle-shaped spot extending from the last of the soft dorsal rays to the very root of the caudal and covering the body from dorsal to ventral median lines. This was lacking, however, in one specimen about 90 mm. long which, nevertheless, was undoubtedly *sellicauda—sella-cauda,* saddle-tail. About midway between lateral line and hinder base of soft dorsal was a roundish black spot on either side, considerably larger than that shown by Evermann & Marsh in their figure. This sometimes extended on to the base of the soft dorsal. Instead of the dark bar on the operculum as shown in the figure referred to, 6 of my specimens had each a distinct round spot on the operculum, while the smallest of all (28 mm. over all) had this elongated into a bar. Nearly all of them had a smaller and less distinct dark patch below and slightly forward of this. The eye was large and had extending horizontally through it a black bar of the exact width of the pupil; the arcs above and below this were white. For this see Plate II, figure 5. This black bar disappeared in every specimen in a few minutes after death. This seems to have some connection with the black spot on the opercle, for in the 28-mm. specimen the spot was elongated into a bar continuous with this black bar across the eye. Other and larger specimens had numbers of black dots between the opercular spot and the dark bar of the eye. From these phenomena one is led to think that as the fish grows older the bar on the opercle loses its connection with the eye and only the spot is left. This small specimen also had a deep red bar across the snout just above the maxillary extending from eye to eye. It likewise had the tips of the highest rays of the soft dorsal and the extreme tips of both lobes of the caudal dark in color.

This elegant little fish was first described and figured by Evermann & Marsh (1900) from a single specimen, 1.63 inches long, from Porto Rico. They give its colors from life, but, as noted above, these do not agree with those of my seven specimens, and their figure must undoubtedly have been made from the dead fish. Jordan (1904) first noted the occurrence of the fish at Tortugas and, later (1905), he and Thompson, having many specimens, corrected Evermann & Marsh's error in figuring the caudal as truncate when it is bilobed. Thompson's field notes also make the base of the second dorsal dark and the tips of anal and caudal fins dark.

21. *Apogonichthys puncticulatus* Poey. Jordan & Evermann, I, 1896, p. 1111; Jordan & Thompson, 1905, p. 239.

I have had but one specimen of this fish but as it differs markedly from that listed by Jordan & Evermann, the data concerning it will be given in full. Jordan and Evermann never saw this fish but list it from Poey (1867). This much by way of explanation.

Length over all 46 mm.; to root of caudal 35 mm.; depth 12 mm.; diameter of eye 4.75 mm.; length of head 15 mm.; of snout 4 mm.; of pelvic fins 13 mm. The fin formula agrees with Jordan & Evermann, but the

scales on the lateral line were unfortunately not counted. The head was contained in the length 3 times (J. & E. 3.75); the depth in the length 3.9 (3.75); the eye in the head 3.2 (2.5); the snout in the eye 1.2 (2).

There were also marked differences in color. Their fish was "reddish with blue reflections on the opercle," mine in life was brownish with a silvery sheen, especially marked on the opercular region. The fins were dark and all closely covered with fine dark spots except the pectorals which were colorless and transparent, save for their blackish anterior or dorsal edges. The bases of the soft dorsal and of the anal and pectoral fins were brown and there was a dark brown patch under the eye. The pelvics were inserted directly under the pectorals and were enormously enlarged, fan-like when hanging down expanded, and reached back to the middle of the anal. The caudal had a distinct though small rounded notch in the middle, as shown in Plate II, figure 6. The scales were large and everywhere covered by fine spots, hence the name *puncticulatus*. The spots on the body and fins brown and closely clustered, making the scales brown and hard to define, and giving the body a brown color. The lateral line was very marked, the pores were large and filled with bubbles while the fish was being handled. These pores communicated with or formed parts of a tube. The bubbles could be forced by the fingers for several millimeters until they emerged at the end of the lateral line.

This fish was taken in an interesting way. We were searching for live conchs *(Strombus bituberculatus)* in shallow water (3 feet) on the southeast side of the Marquesas atoll, some 20 miles west of Key West. The conchs when picked up were thrown into the bottom of the boat and no further attention was given them. One, however, happened to land mouth upward, and in the water filling it this little fish was presently found swimming.

Particular attention was then paid to the position of the *Strombus* shells in the eel-grass, and scores were noted, all with their mouths downward. From all these data the conclusion was drawn that this fish is a symbiont, living normally in the vestibule of this gastropod. The little fish was kept in an aquarium for some days, but was very shy, remaining in hiding all the time. However, it was a very good swimmer when aroused, and was then, with its widespread black pelvics, a striking object.

Undoubtedly this fish seeks shelter and safety inside the mantle cavity of its host, but one has no answer to a number of questions which arise—What benefit does the conch gain? Does the fish come and go at will? When does it feed? Does it stay close at hand or wander far from its host? Does it always return to the same host? And particularly, where does the fish breed? As to the first, it is probable that the conch receives no benefit whatever; at least I can conceive of none. As to the last, it seems not improbable that, like many of its kindred, this fish practices oral gestation.

Numerous instances of this mutualism were reported from the Bahamas by L. Plate[1] in 1908 between a fish of this genus, which he called *Apogonichthys strombi* n. sp., and *Strombus gigas*. His specimens came from water 2.5 meters deep. The conchs at first closed their operculums tightly, but on being undisturbed would relax and allow the fish to slip out into the water at the bottom of the boat. Plate occasionally got two fish from the same conch. If conch and fish were placed together in an

[1] L. Plate. *Apogonichthys strombi*, n. sp., ein symbiotisch lebender Fisch von den Bahamas. Zoologischer Anzeiger, 1908, Bd. 33, pp. 393-399.

aquarium, the fish would presently be found in the mantle cavity, but he was never able to detect the animals in the act of entering. In conclusion Plate raises similar questions to mine and concludes "All these questions might easily [sic] be settled at the Biological Station at the Tortugas."

In this connection it may be well to add that during the summer of this discovery there was at Tortugas another investigator who opened large numbers of a huge *Strombus* of another species, but although close watch was kept no fish was ever found. However, Jordan & Thompson (1905) report a specimen of this fish taken at a depth of 35 feet in West Channel with dynamite. This was light pink in color with a silvery sheen. The dots were most numerous above the lateral line and especially forward. Jordan gives this fish the name of *Minorus puncticulatus* (Poey).

Since the above was written, two other investigators (Hildebrand & Ginsberg, Bulletin U. S. Bureau of Fisheries, 1926, vol. 42, p. 210) working at Key West have found several specimens of *Apogonichthys stellatus* (which they make the correct name of Plate's fish) living in the mantle cavity of conchs and in the cavities of large sponges. My own observations, and those of Plate and of Hildebrand & Ginsberg, together with observations of L. L. Mowbray in the Bahamas, have all been brought together in an article recently published.[1]

XI. Family Serranidæ. The Sea Basses.

The Serranidæ, with some 400 species, divided among 70 or 75 genera, comprise one of the largest families of fishes. They are carnivorous, mainly marine, and are found in all warm and warm-temperate waters. A typical fresh-water form is the common perch, and its characters are so well known and so typical of the family that further remarks are not necessary.

In Florida waters this family is noted for the large size and striking markings of the fishes composing it. On a calm day, when the surface of the sea is unruffled, permitting clear vision of the inhabitants at depths as great as 25 or 30 feet, one may take a trip (never to be forgotten) in the lagoons of the Tortugas archipelago. As the gasoline launch at slow speed moves over the reefs a new world is revealed to the onlooker. In water from 15 to 30 feet deep one comes presently upon what looks like a bowl-shaped depression, at the bottom of which are coral heads varying in size from a small hogshead to ones a dozen times larger. Swimming in and out among these rocks and the algæ and corallines, which attach themselves to almost every crack and crevice, will be scores and perhaps hundreds of fish of all sizes and colors. Chief among these, the rulers of the little realm, will be a number of large, strikingly colored groupers or hinds, fishes of the family Serranidæ. So far as my observation goes, generally there will be not more than 3 to 4 large individuals, oftentimes representing as many genera and species, found in such a locality. Of these, none will present a more striking appearance than the genus and species now to be considered.

22. *Epinephelus adscensionis* (Osbeck). Rock-hind, Grouper. Jordan & Evermann, I, 1896, pp. 1152-1153; pl. CLXXXII, fig. 482; Evermann & Marsh, 1900, p. 152; pl. 11; Jordan & Thompson, 1905, p. 239.

One fine specimen of this fish was examined, having the fin formula; D. XI-16; A. III-8; one soft ray less in the dorsal than either of the above

[1] E. W. Gudger. Inquilinism between the Cheilodipterid Fish, *Apogonichthys puncticulatus*, and the Univalve Mollusk, *Strombus bituberculatus*. Zoologica: Scientific Contributions New York Zoological Society, 1927, vol. 9, pp. 194-200, 2 figs.

authors give in their description. However, it should be stated that Evermann & Marsh's elegant colored figure has X. 17; and Jordan & Evermann's line cut XI. 16. The length of the specimen under consideration was 17 inches over perpendiculars and its depth 4.5 inches.

My specimen was somewhat differently marked from that so beautifully figured in the *Fishes of Porto* Rico, and these chief differences will be pointed out. The olive-colored body showed a great number of distinct white splotches. Five of these formed a row from the angle of the opercle to the hinder insertion of the soft dorsal fin. Lower on the side of the body was a similar row of 5. These set out 2 roughly marked-off H-shaped dark areas on the mid-lateral region, the upper horns of each H running up into the spinous and soft dorsal fins. Across the shoulder and neck region from the anterior base of the spinous dorsal to the level of the eyes were 4 dark bars. The pectorals were very thickly spotted all over, even to the very tips, the spots on the basal portion being orange colored. The ventrals were fringed with red. The dorsal, in both its spinous and soft ray regions, was spotted exactly like the body, save that the number of white spots on the base of the hinder part was fewer than in the Porto Rican fish as figured. The caudal peduncle had on the dorsal side under the projecting dorsal rays a large black spot. The fin itself was spotted like the body, the white spots being especially prominent and thickest at the base. On the sides of the base of the caudal there was a well-marked white bar. The iris was a rich golden-yellow.

Evermann & Marsh state that this fish grows to a length of 2 feet and a weight of 15 to 16 pounds. My specimen, then, must have been adult.

23. *Epinephelus striatus* (Bloch). Nassau Grouper. Jordan & Evermann, I, 1896, p. 1157; pl. CLXXXIII, fig. 483; Evermann & Marsh, 1900, pp. 152-153, pl. 12; Jordan & Thompson, 1905, p. 240.

Only one specimen was examined. This was an immature male 20.5 inches long, 6.25 deep, weighing 4.5 pounds. It was so mauled in the trap and in the boat that no attempt was made to compare it critically with the descriptions noted above.

The Nassau grouper is an excellent food fish, and is common throughout the Florida Keys. My specimen was a small one; at the fish market at Key West they often reach a weight of 6 or 8 pounds. Evermann & Marsh say that in more tropical waters they sometimes reach the extreme weight of 60 pounds.

24. *Epinephelus morio* (Cuvier & Valenciennes). Red Grouper. Jordan & Evermann, I, 1896, p. 1160, pl. CLXXXIV, fig. 485; Evermann & Marsh, 1900, pp. 154-155, pl. 14; Jordan & Thompson, 1905, p. 240.

I have had 5 specimens of this grouper, probably the most abundant at Tortugas, all taken in traps or with the grains in 1912. Four of these measured as follows: (1) 18 inches over all; depth 5 inches; (2) 18.25 inches long; 4 pounds in weight; (3) 21.75 inches long; 6.25 deep, 5.5 pounds weight; (4) 23.5 inches over all; depth 7 inches; weight 7 pounds; (5) length 22; depth 7.5; girth 16.5 inches. The fin formulas and markings of these fish agreed so closely with Evermann & Marsh that no notes were made. That these specimens were all young may be inferred, since much longer ones were seen on the reef. Evermann & Marsh say that they grow to a length of 2 to 3 feet and a weight of 20 to 25 and occasionally 40 pounds.

The carnivorous habits of these fish were well shown by dissection of their stomachs. No. 2 had the stomach much distended by 2 half-decomposed fish. No. 3, an immature female, had in the stomach 2 partly digested porgies,

8 and 9 inches long each. No. 5 had an enormously swollen belly, balloon-like, at the anus forming a square outpushing quite as deep as the normal height of the anal fin. Dissection showed a stomach, 8 inches long and 8 inches in circumference, containing an 11.5-inch snapper, 3.5 inches of whose tail protruded from the œsophagus into the mouth of the grouper. This distension is shown in Plate II, figure 7.

Jordan & Evermann's figure, number 485, plate CLXXXIV, was evidently made from a preserved specimen from which the markings had all disappeared. At any rate their figure shows none.

25. *Promicrops guttatus* (Linnæus). Spotted Jew Fish. Jordan & Evermann, I, 1896, pp. 1162-1164, pl. CLXXXV, figs. 487, 487*a*, 487*b*; Jordan & Thompson, 1905, p. 240.

Two specimens were taken on shark hooks by Mrs. E. E. Hayden and family with the help of Captain George Tracy from the *Anton Dohrn* on the night of July 7, 1914. The *Dohrn* was anchored in the channel southeast of Garden Key and about 300 yards from the sally-port of Fort Jefferson. These gigantic fishes had both jaws, the roof of the mouth, the pharyngeals and the inner surfaces of the gill-arches close set with masses of thorn-like teeth. The large, bony, closely imbricated scales made it necessary to use an ax to cut into the fish.

The measurement of the fish are set out in the following table. It should be noted, however, that the fishes were measured while suspended from the davits of the *Dohrn* by ropes run through the mouths and out under the opercula, and hence the mouth of each was distorted and the measurements though relative are not normal.

Measurements of Jew Fish	I		II	
	ft.	*in.*	*ft.*	*in.*
Length, tip of snout to tip of caudal	5	8	7	0
Length, tip of snout to base of caudal	4	6	5	8
Length, tip of snout to base of pectoral	1	9	2	1
Length, tip of snout to base of dorsal	1	9	2	2
Length, tip of snout to base of anal	.....		4	5
Girth, greatest	4	3	4	10
Girth, around gill-covers[1]	4	1	.....	
Girth, around "small" of tail	.....		1	9
Depth	1	9	2	0
Head, across from angle to angle mouth[1]	1	10	1	10
Head, across from eye to eye	0	6.5	0	6.5
Mouth, width across, angle to angle[1]	1	2	1	2
Mouth, vertical gape[1]	0	9.5	0	9.5
Mouth, around curve upper jaw	1	8.5	1	9.5
Dorsal fin, length of spinous part	1	5	2	0
Dorsal fin, length of whole	2	6	2	11
Pectoral, length	1	0	1	2
Pectoral, breadth	0	11	0	11
Pectoral, breadth of base	0	4.5	0	4
Caudal, length	0	11	1	0
Caudal, spread	1	7	1	11

[1] Distorted, measurements approximate only.

Figure 1 on plate IV is from a photograph of this huge fish suspended from a davit on our dock. The figure, being reduced to fit the plate, fails to give an adequate idea of the great size of this our largest sea bass.

A number of specimens were seen around big coral heads, but these 2 were the only ones taken. They were caught on hooks baited and set for sharks, and neither made any appreciable resistance. Their great bulk and dead weight, however, made them difficult to handle. They were caught within 50 yards of each other and were possibly a pair, male and female. Unfortunately, press of work preparatory to a trip to Key West for sharks on the day following their capture prevented my dissecting them.

At Key West, jew fish are much esteemed for food and sell readily, and at a good price. They love holes under wrecks, mangrove roots, and coral heads. The fishermen know their resorts and take them with small shark hooks, and carry them alive to the fish market. I have seen Peter Roberts, keeper of the fish market, with half a dozen tethered out, so to speak, each with a rope through its mouth and gills and tied to a pile. However, to prevent their being eaten by sharks, they are generally kept in large boxes, made of slats to allow easy ingress and egress of water. If left untethered, they would be likely to break the frail slats and escape.

Our specimens were very dark, due possibly to the great number of round black spots found everywhere. Specimens seen at the Key West fish-market wharf were mainly of a muddy brown color.

This huge percoid is very abundant in the Florida Keys, and even larger specimens than those under consideration have been put on record. Jordan & Thompson note from Tortugas an example 7 feet 6 inches long. It grows to a great weight, probably reaching 500 or 600 pounds. Our largest specimen was weighed on a stout spring scale having a circular dial registering up to 320 pounds. The fish, however, forced the recording hand to a point considerably beyond the 320 pound mark—probably it weighed as much as or even more than 400 pounds. I have been told of a specimen, caught at Key West, of 7 feet 2 inches in length and 6 feet in girth, which after being cleaned and having a good portion of the flesh cut off still weighed 410 pounds. The jew fish is certainly the largest teleost taken in Atlantic waters, the giant of the eastern Serranids.

26. *Mycteroperca venenosa apua* (Bloch). Rock Fish, Grouper. Jordan & Evermann, I, 1896, pp. 1173-1174; Jordan & Thompson, 1905, p. 240.

Four specimens were examined: No. 1, 2 feet 4.5 inches long over all, 8 inches in depth, 12 pounds in weight; No. 2, 2 feet 8.5 inches long, 8.75 inches deep, 15.5 pounds in weight; No. 3, 2 feet 8.75 inches in length, 8 inches in depth, 17 pounds in weight; No. 4, 2 feet 9.5 inches long, 9 inches deep, 21 inches in circumference, and 18 pounds in weight. No. 1 was a female with immature sac-like ovaries seemingly divided internally into crypts. Nos. 2 and 3 had the pectorals tipped with yellow, and both had great numbers of crustacean parasites in the mouth, in the external nares, in the folds around the eyes and around the mouth. No. 4 had a 9-inch grunt in its stomach. Its mesentery was loaded with fat, which looked and felt like, and tore with the sound of a pig's mesentery laden with fat. Seventeen pyloric cœca were counted in one specimen. Another had 17 soft dorsal rays instead of the 16 normally present.

27. *Mycteroperca bowersi* Evermann & Marsh. Rock-hind, Rock Fish. Evermann & Marsh, 1900, p. 158, fig. 45.

A large specimen of this fish gave the following measurements: length to tip of caudal 2 feet 10 inches; to base of caudal 2 feet 5 inches; of head only 12 inches; depth 9 inches; girth 1 foot 10 inches; vertical gape 5 inches,

horizontal 5 inches; lower jaw around curve 9 inches; weight 21 pounds. The mouth had 4 big fixed canines spaced one on each side of the median line in each jaw. On each side of each jaw there was an outer row of small teeth. Inside of these in each jaw, but mainly on the lateral front sides of upper jaw and on back sides of lower were many conical teeth hinged so as to be depressible backward, forming a very efficient swallowing apparatus.

This fish, taken in a trap on the west side of Loggerhead Key, was put in an aquarium over night, but died. When dead its mouth and gills were widely distended, making a cavity half the size of a water bucket or larger. On the following day another large specimen was taken in a trap but escaped overboard in the process of being taken out.

The colors agreed closely with Evermann & Marsh's description, as did the comparative measurements. Judging by these specimens, their figure is an excellent one. They named this new species for former Commissioner of Fisheries Bowers. This fish is new to the Tortugas and to the waters of the United States.

28. *Hypoplectrus unicolor puella* (Cuvier & Valenciennes). Jordan & Evermann, I, 1896, pp. 1189-1192; Jordan & Thompson, 1905, p. 240.

Jordan & Evermann say of this genus of Serranids that "a study of their relations offers many difficulties," that the large number of species is "nominal," and that there is probably but one species with many variations in color—*H. unicolor*. This conclusion is borne out by the experience of Jordan & Thompson with their 3 specimens of *unicolor* (variety not specified). My one specimen, taken in a trap at Loggerhead Key, in July 1912, offered so many difficulties that to make sure of it I sent it to the United States National Museum, where Mr. B. A. Bean determined it as *Hypoplectrus unicolor* variety *puella*.

The fin-ray count for this fish was D. X, 16; A. III, 7 (8 ?); while Jordan & Evermann give D. X, 14 or 15; A. III, 8. This last count, as in scores of other cases, depends on whether the last ray counted is a true ray arising from a root of its own, or is merely a branch of the last true ray. In their figure of *H. unicolor nigricans*, which according to the text has 8 rays, the eighth ray is as plainly the hinder half of the split seventh ray as is the similar structure in their figure of *H. gemma*, to which they correctly assign 7 rays in their text. (See their volume IV, 1898, plate CLXXXIX, figures 496 and 497.)

The color variations of this fish are extreme. Jordan & Evermann say "Coloration extremely various," species "nominal." Jordan & Thompson had 3 specimens from the Tortugas, each unlike the others in color pattern. My one specimen was somewhat like their third. Its ground-color was on head and pectoral region a bright yellow, the remainder of the body was brassy-yellow. A faint blue band ran around the upper jaw. Eye surrounded by a bright blue circle, as shown in Plate II, figure 8, and as noted by Jordan & Thompson. Top of head marked off with short blue bars as shown in the sketch. Back of the eye 3 lighter blue bars extended vertically across the opercle and continued up on head and shoulder as lavender-blue. A fourth stripe barely touched the hinder edge of the opercle. On the body about 21 lavender-blue bars stood in a nearly vertical position (slightly pointing forward above), reaching into the dorsal above and ending on the belly below. Bar one, however, ended in the axil of the pectoral, and some bars on the central part of the side did not extend all the way down; further there were a few short intercalary bars, about one-third the width of the

others, found scattered among the others, and on the caudal peduncle there were 2 short bars close to the caudal, partly obscured by a large glossy black spot which covered both the dorsal and lateral parts of the base of the caudal. A distinct offset from this patch gave the lower half of the caudal fin a distinctly lighter color than the upper half. The dorsal fin was a bright yellow, broken up in a somewhat zigzag fashion by the continuation of the lavender bars. The pectorals and the caudal showed a faint pink, and the pelvics and the base of the anal were a bright lemon-yellow. Altogether this fish presented a striking appearance. Unfortunately, no other was ever taken.

XII. Family Priacanthidæ. The Catalufas.

This small family of tropical percoids is composed of carnivorous fishes, mainly inhabitants of deep waters, and generally of reddish or rose color. The family is represented in the Tortugas by one genus and species.

29. *Priacanthus cruentatus* (Lacépède). Big-eye. Jordan & Evermann, I, 1896, p. 1238; Evermann & Marsh, 1900, p. 167; Jordan & Thompson, 1905, p. 241.

Several specimens were taken with dynamite on Loggerhead Reef, and 2 were measured and studied. No. 1 measured 8.75 inches to tip of caudal; 7.5 to base of caudal; head only 2.4; eye (diameter) 1; depth 2.9 inches. No. 2 was 7.9 inches over all; 6.8 to base of caudal; head only 2; eye (diameter) 0.8; depth 2.6 inches. The fin-ray counts were: D. X, 13; A. III, 14. These agree with the authorities.

The mouth was considerably protractile, and when protruded gaped widely. In gaping thus, the lower jaw remained nearly horizontal, while the upper stood almost vertical. Mouth with large free tongue, and with breathing valves above and below—the upper being the larger. Back of mouth, including infra-pharyngeals, red. Gill-rakers on first arch long and slender, pointing forward and downward.

It is difficult to say whether this is a red fish painted with silver, or a silvery fish which has been dipped in red; probably the former is more correct. Perhaps the most prominent features of the fish are the enormous eyes, which are nearly equal in diameter to half the length of the head. These great eyes were provided with an adipose sheath. The anterior nasal opening was very small, and compared to it the posterior was an enormous chasm as shown in Plate II, figure 9. The expanded dorsal and anal fins were very striking, the fin rays showing very prominently in the membrane, but unfortunately no note was made of their color. The dorsal spines were staggered and depressible in their groove. The ventrals were joined to the belly by a membrane.

Comparison of these fish with Evermann & Marsh's colored plate of *P. arenatus* shows that *P. cruentatus* is not merely a very symmetrical and beautifully colored fish, but that it is more ovate than the other, with smallish trim peduncle, a higher back and more curved (not flat) forehead—a trimmer fish than *P. arenatus*.

XIII. Family Lutianidæ. The Snappers.

This large family of active, voracious, carnivorous, marine percoid fishes is represented in the Tortugas by 8 species according to Jordan & Thompson.

Of these I have studied 4 species; 3 belonging to the genus *Neomænis (Lutianus)*, and one to *Ocyurus*.

30. *Neomænis griseus* (Linnæus). Gray Snapper, Mangrove Snapper. Jordan & Evermann, II, 1898, pp. 1255-1256; Evermann & Marsh, 1900, pp. 170-171, pl. 17; Jordan & Thompson, 1905, p. 241.

Specimens were examined and measured as follows: No. 1, 13 inches long over all, 3.75 deep, weight 0.5 pounds; No. 2, 17.75 inches long, 5 deep, 2.25 pounds in weight; No. 3, 17.75 inches long, 4.75 deep, 2.25 pounds in weight; No. 4, 20.75 inches length over all, 17.75 to base of caudal, head alone 6 inches, diameter of eye 1 inch, depth 5.5 inches, girth 12.5 inches, weight 3 pounds. There was no departure from the normal fin-ray formulas. One of these fish was a female having ovarian eggs, each with a large germinal vesicle and a nucleolus nearly as large as the vesicle.

On July 25, 1914, a number of *N. griseus* and *N. apodus* were taken together with dynamite at a coral head on the west side of Loggerhead Key. They were brought in at once and compared. Six specimens of *griseus* each had one large canine in front on each side of the upper jaw. The lower jaw fitted within the upper and had slight notches on the edge for the upper canines, which showed on the outside when the mouth was closed. The snout and head of *griseus* were notably more pointed than in *apodus*, the body longer and trimmer, and the pectorals narrower. The eye was slightly ovoid and pointed forward.

The color of this snapper seen swimming in the water was a distinct *gray*, differing markedly from the descriptions given in the *Fishes of North America* and the *Fishes of Porto Rico*, and quite unlike the color shown in Evermann & Marsh's plate. A blackish band ran obliquely upward and backward along the side of the head and through the eye. In one Tortugas specimen there were 8 black spots on the lower and inner half of the left pectoral, and 4 on the middle of the corresponding right fin. There was a dark spot on the left gill-cover, a like spot on the tenth soft dorsal ray, and a small one at the base of the lower half of the caudal.

The gray snappers have some very interesting habits.[1] Some two or three dozen used to hang around the western dock at Loggerhead, feeding, playing and seemingly resting under the shadow of the dock, but mainly they were there to feed on scraps thrown overboard by the cook. The fish apparently knew this individual and manifested no fear of him whatever, for whenever he appeared carrying a bucket or pan the gray snappers were all eagerness, and as he raised his hands with this utensil there was a grand rush and the scraps and snappers arrived at the surface at the same time.

However, let any one else walk out on the dock (which was about 8 feet above the water) and some of the snappers (generally those nearest) would turn slightly on one side, thus keeping a cool and wary eye on the intruder. If he stooped to pick up something, the snappers would move off, and if the object picked up was the grains (a pair of which was nearly always kept at the pier head) the less bold fish would depart for deeper water. However, the bolder seemed to like the game and held their ground until the grains were thrown, when, in a flash, they were gone. Not one, so far as I can recall, was ever taken thus, though our most expert strikers were continually practicing on them. Some of the fish presently seemed to recognize that it was a game and would hardly move six feet away when struck at.

[1] A brief account of these habits has been published in *Natural History*, 1923, vol. 23, pp. 252-253, under the title—"Notes on the Behavior of the Gray Snapper, etc."

They were also much tried for with hooks, especially by the newcomers, to whom they were an ever-present temptation. For years unsuccessful efforts were made to hook them, but none was taken in this way until the summer of 1915 when our cook's assistant, a professional fisherman from Key West, used occasionally to hook one at night. Several, however, were taken at one time by surrounding them with a seine.

Our favorite bathing place was a sandy beach on the east side of the island directly in front of the station. Here a dock, which ran out into water 5 or 6 feet deep to support the intake pipe for the pump, acted as a spring board for diving and as an observatory for watching the fish. Around this dock and in and around the reef of coral limestone, which extended from it roughly parallel with the shore, large numbers of fishes played, presently becoming quite tame. Among these fishes were probably 100 gray snappers which had collected, seemingly out of sociability for themselves and for us. When the other men had swum away I used frequently to stand quietly on this ledge of rock, whereupon the gray snappers would swim all around me, playing with each other and seemingly with me, for they would often come within a foot of me, so close that frequently I expected them to nibble at my fingers and toes. Then when I turned and swam toward shore a band of them on either side would fearlessly accompany me into water knee-deep. This happened not once but a score of times.

Gray snappers are fond of ghost crabs *(Ocypoda arenaria)*, and used to catch them in the following fashion. It was our custom after supper to walk to one end or the other of the island. Loggerhead Key is in the shape of a flat crescent with the concave side toward the west. The best walking was on the hard sand between high- and low-water marks on the western side. As we walked along on land the gray snappers would parallel our course in the water, and when, as frequently happened, ghost crabs, frightened by our approach, scuttled into the water, there was a quick rush by the snappers, and the crabs never came back. In this way the snappers got their suppers.

It is interesting to note that in these bands the fish were nearly all of the same size. While there may have been some difference of size (2 or 3 inches perhaps) there were never any small snappers, and indeed no small ones were ever taken.

The specific name describes their color—gray. They are also called mangrove snappers because they lurk in the tangles of mangrove roots, probably to catch the crabs found on and among these.

31. *Neomænis apodus* (Walbaum). Schoolmaster. Jordan & Evermann, II, 1898, pp. 1258-1259, pl. CXCVII, fig. 515; Evermann & Marsh, 1900, pp. 172-173, pl. 19; Jordan & Thompson, 1905, p. 241.

Numerous specimens were examined and 2 measured. Of these, No. 1 was 9 inches long between perpendiculars and 2.75 deep; while No. 2 was 10.75 inches long and 3.25 deep. Fin rays were D. X-14; A. III-8, as called for by the authorities. Evermann & Marsh's colored figure, however, has 9 plain soft rays in the anal and either 14 or 15 in the soft dorsal—the count is rather difficult to make.

The colored figure referred to has a bluish V on the nape; back of it, beginning midway between the V and the first dorsal spine, are 9 vertical bluish bars with the rudiment of a tenth at the base of the caudal. Both texts and also Jordan & Evermann's figure call for 8. The two Tortugas

specimens carefully noted for color showed 8 bars. However, there must be considerable variation in this fish—a 9-inch Porto Rican specimen had "about 12 faint brassy lines running backward and somewhat upward." The Tortugas specimens were somewhat darker in the dorsal region than is shown in the colored figure referred to.

As to the teeth, one specimen had but 1 left upper canine, another had 2, and a third had a large upper canine on each side, another 2 on each side—the inner being the larger. The lower jaw fits inside the upper and has depressions or notches on each side to receive these large canines. The head and snout are blunt and heavy.

This is probably the handsomest and most striking of the snapper tribe found in the Tortugas, where it is very abundant.

32. *Neomœnis analis* (Cuvier & Valenciennes). Mutton Fish. Jordan & Evermann, II, 1898, pp. 1264-1265, pl. CXCVIII, fig. 517; Evermann & Marsh, 1900, pp. 176-177, pl. 21; Jordan & Thompson, 1905, p. 241.

One specimen was examined and measured: total length (outside) 2 feet 5.5 inches; length to base of caudal 2 feet; depth 8.5 inches; girth over fourth dorsal spine 1 foot 8 inches; weight 15.5 pounds. Maxillary reaching only halfway to front margin of eye. Iris fiery red, even in the dead fish. Oral breathing valve across floor of mouth in front. Lobes of caudal about equal as shown in Evermann & Marsh's colored plate, not upper lobe markedly the longer as in Jordan & Evermann's figure (Evermann & Marsh, however, say upper lobe longer).

Color very much as in Evermann & Marsh. However, no upward and backwardly directed stripes were visible, nor any vertical bars. These authors say "about 6 dusky bars crossing the sides vertically," but their colored figure shows about 9. The dorsal edge of the caudal was blackish but there was no dark margin behind. On each side, the dark blotch was found one scale above the lateral line and it covered 2 scales just under soft dorsal rays 1 and 2. Across the base of each pectoral was a dark olive bar.

This fish was a female, having paired ovaries confluent into a common duct leading to the genital papilla situated just behind the vent. The lobes of the ovaries were 4 inches long and 1.25 inches in diameter. The eggs were very small and were evidently unripe. In the mouth were many crustacean parasites, seemingly of the same kind as those noted as infesting *Mycteroperca venenosa apua*. The œsophagus led into a large bag-shaped stomach very similar in appearance to a short and stout liquid CO_2 bottle. The stomach was 7 inches long and 8 inches in circumference, and contained a 10-inch grunt with the tail doubled so as to fit in. On the right of the stomach and about midway, there arose the small intestine, with its well-marked pyloric constriction but a short distance removed from the beginning of the tube itself. For these structures see Plate II, figure 10.

The specific name of this fish refers to the markedly long, pointed and prominent anal fin. This fin, according to both sets of authors, should have 8 soft rays, but Evermann & Marsh's colored figure shows 9. This is a very good food fish and since it is abundant about Key West it forms no inconsiderable source of the food supply.

33. *Ocyurus chrysurus* (Bloch). Yellow-tail, Rabirubia. Jordan & Evermann, II, 1898, pp. 1275-1276, pl. CXCIX, fig. 520; Evermann & Marsh, 1900, pp. 180-181, pl. 23; Jordan & Thompson, 1905, p. 241.

Of this abundant snapper my best specimen was caught by John Mills while trolling between Loggerhead and Bird Keys. Its total length to tip of caudal was 1 foot 11.5 inches; to fork of caudal 1 foot 7.5 inches; to base of caudal 1 foot 5.5 inches; its girth 1 foot 1.5 inches; its depth 6 inches; and its weight 3.5 pounds. Its fin-ray count was D. X, 13; A. II, 10. The authorities above noted agree on D. X, 13; A. III, 9. Evermann & Marsh's figure, however, has it D. X, 14; A. III, 10. Jordan & Evermann's has D. X, 14; A. III, 9. One of my specimens also had 9 rays in the soft anal. The eye was markedly large, measuring 0.75 inch in diameter and the prominent pupil 0.44 inch.

With reference to the internal organs, but few notes were made. The tongue was long and free for a good part of its length, and oral breathing valves above and below were found. The liver was very small for a carnivorous animal, 5 or 6 cœca were found, and the wall of the intestine was noted as being very thin. Posteriorly there was a short, bifurcated, post-anal extension of the abdomen running back to about the roots of the fifth and sixth anal rays and containing nothing but the 2 short horns of the air-bladder.

The colors of the fresh specimen were about as follows: beginning on the snout a bronzy-green band extended backward through the eye, across the opercle, down the mid-lateral region widening out under the hinder two-thirds of the soft dorsal, where it crossed the lateral line and, under the overhang of the fin, joined the like band from the other side to cover the top and lateral two-thirds of the caudal peduncle. Above this bronzy band the ground-color was a pale violet or lavender, below this orange-red, grading into white on the belly. The edges of the scales were marked off in violet, brighter above the band, fainter below, giving the side of the fish a curious lattice-work appearance, better indicated in figure 520 (J. & E.) than in plate 23 (E. & M.). Above the bronzy band a number of small spots of like color decorated the scaly opercle and head, while on the side a number of large spots of the same color were found, three of these being below the lateral line and one on it, while a larger number was noted between this line and the base of the dorsal. Below the mid-lateral band were several faint and narrow bands of the same color extending from the base of the pectoral backward to the anal region and on to the caudal peduncle. They were so faint that it was hard to make out any definite number. Certainly there was not any such number as the 8 referred to by our authorities as alternating with an equal number of pinkish stripes. It is interesting to note here that plate 23 (E. & M.) shows but 5 of each kind.

The lower jaw and lower part of the opercle varied in color from pinkish to orange. The dorsal fin was a lemon-yellow, anal a lighter yellow. Pectorals were green on upper outer side of base, yellow on inner side of base; the upper half of the outer fin-rays was straw-yellow, lower half on outside colorless. Pelvic fins were yellowish-green on outer (front) rays, hinder or inner rays colorless.

On one calm afternoon in July 1913, I circumnavigated the southeastern end of Loggerhead Key in one of our launches at slow speed. Great numbers of fishes were seen, yellow-tails most abundantly—there being probably 10 times as many of them as of all other fishes together. When they swam under me or in any way close to the perpendicular, their yellow dorsal fins made a golden streak down the central dorsal violet region, and this in turn was flanked by the greenish-bronzy lateral bands on each side.

This genus of snappers, *Ocyurus*, has but one species, *chrysurus*. It is said to grow to a length of 2 feet and a weight of 3 or 4 pounds, and my 23.5-inch, 3.5-pound specimen was then full-grown. At Key West, in the summer, when neither Spanish mackerel nor pompano are to be gotten, the yellow-tail is the most toothsome fish served at restaurants and hotels.

XIV. Family Hæmulidæ. The Grunts.

One of the most numerously represented families of fishes at the Tortugas is that of the Grunts, numerous both in genera and species and innumerable in individuals. Their common name is given because of the characteristic noise made by means of the air-bladder. The scientific name is derived from 2 Greek words meaning red gums and probably has been given because so many of these fishes have red mouth-cavities. The grunts are small, carnivorous fishes, very abundant and very palatable, hence they form an important source of food for the inhabitants of the Florida Keys and the West Indies.

34. *Hæmulon macrostomum* Günther. Gray Grunt, Striped Grunt. Jordan & Evermann, II, 1898, pp. 1296-1297, pl. ccxv, fig. 529; Evermann & Marsh, 1900, pp. 186-187, fig. 51; Jordan & Thompson, 1905, p. 242.

Numerous specimens were examined, varying from 6 to 11.5 inches in length between perpendiculars. All the authorities quoted above give fin formulas D. XII, 16; A. III, 8. My specimens agreed as to dorsals, but all had 9 soft rays in the anal. The figures referred to above (which are identical) have 3 spines plus the rudiment of a fourth and 9 soft rays in the anal.

This fish had a large tongue, and also oral breathing valves above and below to prevent regurgitation of water in the process of breathing. The dorsal spines were set staggered in their sheath, so that when depressed they might fit perfectly in their groove. The first anal spine was very small, almost rudimentary. There was a deep cleft between the upper jaw and the cheeks, and on the lower jaw were 1 median and 2 lateral pores of considerable size. The fish, on being transferred from one aquarium to another, justified its name by grunting loudly.

The general color of these specimens was as given in Evermann & Marsh. In addition, underneath the spinous dorsal there was a bright greenish-brassy region. The dorsal lobe of the caudal had something of the same color, and on the sides of the body many scales showed a yellowish-brassy tint. The bases of the soft dorsal, the anal, and the caudal were greenish-yellow. The head parts and the under sides of the body were a dark micaceous. The lateral streaks or lines referred to by both sets of authorities, but only partly shown in their common figure (which was probably drawn from a preserved specimen), were very marked. I found 9 bars, and, above the wide median lateral one which ran directly backward from eye to root of caudal (Jordan & Marsh's number 5 but number 4 on my specimens), a collection of irregular dark markings—evidently corresponding to a stripe. This description is from a formalin specimen checked, however, by a living fish in an aquarium. The longitudinal stripes below the eye were visible on the formalin specimen but were indistinctly seen on the fish in the aquarium. Either these stripes are under the nervous control of the fish or else there is great variation—probably both. The formalin specimen had an inky-black spot on the lower edge of the opercle, partly covered by the preopercle.

35. *Hæmulon parra* (Desmarest). Sailor's Choice. Jordan & Evermann, II, 1898, pp. 1297-1298, pl. ccIV, fig. 530; Evermann & Marsh, 1900, pp. 187-188, fig. 52; Jordan & Thompson, 1905, p. 242.

Of this abundant grunt but one specimen was examined. This was 9.9 inches long over all and 8 to the base of the caudal. Its fin formulas were: D. XII, 17; A. III, 11. Evermann & Marsh's are D. XII, 17; A. II, 8. Their figure, however, has D. XII, 18; A. III, 8 or 9 (the drawing is not clear). Jordan & Evermann give D. XII, 17; A. III, 7; and their figure (the original of Evermann & Marsh's) shows of course the same discrepancy as above.

My specimen agreed closely with the description given in the *Fishes of Porto Rico*. However, the head was not dark but light as shown in figure 52 (E. & M.) referred to. The preopercle is sharply serrate as shown in this figure, though the description on page 187 says "wholly without serrations." Jordan & Evermann say "finely but sharply serrate." However, they add that with regard to serration on the preopercle there is much variation. Specimens from Havana are strongly serrate in some cases, little in other; Key West forms are rather intermediate. The dorsal spines were set staggered so that when depressed they would pack smoothly in their groove. The back was more steeply arched than in the figure referred to.

36. *Hæmulon carbonarium* Poey. Ronco Carbonero. Jordan & Evermann, II, 1898, pp. 1300-1301; Evermann & Marsh, 1900, pp. 188-190.

One specimen only taken, locality not noted. Length over all 9.75 inches; to base of caudal 8.5; depth 3.12; length of head 2.75; of snout 1.12; diameter of eye 0.75 inch. Fin-ray count: D. XII, 16; A. III, 8, agreed absolutely with the authorities above quoted. Dorsal spines set staggered in their groove. Pectorals falcate, reaching about to vent. Pelvics with median accessory scale.

Color in general as given by Jordan & Evermann. Fourteen brassy bands on the sides, some extending full length of body, some ending about halfway back. Caudal darkish, tips of pectoral and pelvic fins and of soft dorsal and anal also somewhat dark.

This seems to be the first capture of this fish in the waters of the United States.

37. *Hæmulon sciurus* (Shaw). Yellow Grunt, Striped Grunt, Bear Grunt. Jordan & Evermann, II, 1898, pp. 1303-1304, pl. ccv, fig. 531; Evermann & Marsh, 1900, pp. 189-190, fig. 53; Jordan & Thompson, 1905, p. 242.

One young and four adult specimens were examined and all gave the same fin-ray count: D. XII, 16; A. III, 9. These counts agree with the figures above referred to, but disagree with their authors who assign only 8 rays to the soft anal. These adult specimens ran from 10 to 11 inches in length. There is much variation in the number of the bright, brass-colored bands or stripes on the sides. One of my specimens had 15 in the shoulder region whereas a little further back only 12 could be counted. Another had 13 only. Evermann & Marsh say "about 10," while Jordan & Evermann note "about 12." These brassy bands alternate with about an equal number of blue stripes, all of which taken together give the fish a striking appearance. It is perhaps more correct to say that the general color of the fish is a brassy-yellow with blue stripes, etc.

A young specimen, 57 mm. long and 17 deep, had 7 blue bands across the head and anterior part of the body. Two of these crossed the eye, the

lower one forking and sending backward 2 branches. Betwixt the lateral line and the base of the dorsal was a very bright brassy band. At center of base of caudal was a large dark spot. Both references above speak of the adults having a dusky area at base of caudal, but their figures (which are identical) do not show this, nor was it noted on any of my specimens. The lateral line made a rather abrupt bend downward under hinder base of soft dorsal. The foregoing points were all verified on another young *sciurus* of about the same size living in the aquarium. This was a very lively and interesting pet.

38. *Hæmulon plumieri* (Lacépède). Common Grunt. Jordan & Evermann, II, 1898, pp. 1304-1305, pl. ccv, fig. 532; Evermann & Marsh, 1900, pp. 190-191, fig. 54; Jordan & Thompson, 1905, p. 242.

Of this common fish 6 specimens have been had, ranging from 8 to 9.25 inches in length over all. Notes were made on 2 of these. The fin-ray count on the best specimen was D. XII, 16; A. III, 9 or 10—depending on whether the last ray is counted as 10 or as a part of 9. There seems to be some variation in this fish, for Evermann & Marsh give D. XII, 15 or 16; A. III, 8 or 9; while Jordan & Evermann make it D. XII, 16; A. III, 8; and the figure, which the former copy from the latter, contains 17 soft dorsal rays and 9 anal.

On my best specimen 9 blue bands crossed the forehead between top of eyes and snout. On the right side of the head there were 12 blue stripes, 3 of which intersected the orbit of the eye. These stripes showed much more plainly on the operculum than appears in the figure referred to. On the body just back of the gill-openings faint traces of these stripes could be noticed. These are rather indefinitely referred to by the authorities quoted; probably they are lacking in many specimens. Evermann & Marsh speak of broad brassy lines extending from the opercle on to the body, and in one of my specimens these ran obliquely upward to the lateral line and thence backward parallel with this. The lateral line, at a point where it intersected a line joining the hinder edge of the soft dorsal and the anus, made an abrupt bend downward, not the gentle curve found in the figure referred to. See my Plate II, figure 11. On one occasion some specimens of *sciurus* and *plumieri* were brought in together. The differences between them struck the eye at once. One of the latter was a female with immature ovaries.

39. *Brachygenys chrysargyreus* (Günther). Jordan & Evermann, II, 1898, pp. 1307-1308, pl. ccvi, fig. 533; Jordan & Thompson, 1905, p. 242.

This fish was 7 inches long over all; 5.75 to the base of the caudal, and 1.75 inches in depth. It differs from Jordan & Evermann's description in relative proportions and in having one less soft dorsal ray. The interorbital space goes 3.2 times into the head instead of 4, and dorsal spine IV. 1.9 times instead of 1.75. This fish in all other points agrees with the excellent description referred to. Only one specimen was taken, though Jordan & Thompson (1905) record it as being common.

40. *Anisotremus virginicus* (Linnæus). Pork Fish. Jordan & Evermann, II, 1898, pp. 1322-1323, pl. ccix, fig. 539; Evermann & Marsh, 1900, pp. 195-196, fig. 57; Jordan & Thompson, 1905, p. 243.

Only one specimen of the pork fish has been examined, although it is very abundant. This fish was 6.5 inches long over all; 5.5 to root of caudal;

depth 2.5. Jordan & Evermann give the fin formulas D. XII, 17; A. III, 10 or 11. Evermann & Marsh D. XII, 16; A. III, 9. My specimen showed D. XII, 16; A. III, 10. The pectoral fins are slightly longer than the head and their tips reach back to anal spine II. This was 25 mm. long as contrasted with dorsal spine II, 21 mm. in length.

The coloration of my specimen was quite different from Evermann & Marsh's, which in turn does not agree with their figure. The sides of the Tortugas specimen had 7 longitudinal yellow bars. Of these, Nos. 1 and 2 on each side united on the median dorsal line under spine VIII of the dorsal fin; while 4 and 5 of each side coalesced in similar fashion under the soft dorsal. All the lateral yellow bars and not "the upper 3 or 4" were forked anteriorly. Bar 6 was composed of 2 rows of yellow scales separated by a row of silver scales as far back as the origin of the soft dorsal, where the yellow scales united to form a yellow bar which, under the hinder edge of the soft dorsal and thence to the base of the caudal, was divided into halves by the lateral line which ended in the spines of the caudal. In the Porto Rico figure the lateral line is below this bar.

The vertical black bar from the origin of the spinous dorsal, when it reached the base of the pectoral, turned forward and downward at an obtuse angle and followed the branchiostegal membrane, ending on the isthmus. This is referred to in the description of the Porto Rican fish, but is not shown in the figure. The black bar running from nape through eye to angle of mouth was present, as was also another crossing the head transversely between the eyes. This is neither described nor figured. The upper part of the spines and the interspinous membrane of the first dorsal were dotted with black. The pelvics were yellow overlain with a thick, black stippling. The bars only of the pectorals were black stippled.

It is to be regretted that there is no adequate figure of this fish. A photograph, unless made under exceptional circumstances, would hardly bring out the points enumerated above.

XV. Family Sparidæ. The Porgies.

These fishes, of which the Sheepshead, Pin Fish and Scup are common northern representatives, are rather short, heavy-set, deep-bodied fish with strong dorsal spines and steeply sloping foreheads. Four genera, *Archosargus, Calamus, Diplodus* and *Lagodon,* are represented in the Tortugas. I have studied species of the second and third genera only.

41. *Calamus calamus* (Cuvier & Valenciennes). Saucer-eye Porgy. Jordan & Evermann, II, 1898, pp. 1349-1350, pl. ccxii, fig. 546: Evermann & Marsh, 1900, p. 201, fig. 58; Jordan & Thompson, 1905, p. 243.

This species is very near *C. proridens* and the identification was for some time in doubt, but on the whole its characters agreed more closely with those given for *C. calamus.* In my specimen the hindermost teeth of the innermost row were notably enlarged. The curve of the forehead showed a slight hump between the eyes.

This specimen was small, the total length between perpendiculars being 9.75 inches, of which the caudal alone measured 2 inches, depth 3.5 inches. The fish seems to be of small size, since the largest specimen ever taken, was 15 inches long. Its weight reaches a pound or more. It is an

excellent food fish. The present specimen was taken in a trap on Loggerhead Reef.

42. *Calamus bajonado* (Bloch & Schneider). Jolt-head Porgy, Bajonado. Jordan & Evermann, II, 1898, p. 1352, pl. CCXIII, fig. 548; Evermann & Marsh, 1900, p. 202, pl. 25; Jordan & Thompson, 1905, p. 243.

One specimen only of this porgy was studied. Its length over all was 9.25 inches; to base of caudal 7.75; depth 3.5 inches. The head was 2.1 long, the eye 0.7 inch in diameter. The dorsal spines were set staggered in their groove, practically disappearing when depressed. The pelvic fins had median accessory scales and on the outer side of each fin were notably long scales. The pectoral fin was long and somewhat falcate, reaching to base of anal.

This fish is known to attain a length of 2 feet and a weight of 8 to 10 pounds. The specimen under consideration, which was taken with dynamite off Loggerhead Reef, was young, probably not over a year old. Presumably this fish is not uncommon around the reefs.

43. *Diplodus holbrooki* (Bean). Spot-tailed Pin Fish. Jordan & Evermann, II, 1898, p. 1362, pl. CCXVII, fig. 555; Jordan & Thompson, 1905, p. 243.

Two specimens of this fish were examined, though several were taken in 1913 and 1914. Both had fin rays: D. XIII, 13; A. III. 13, agreeing closely with Jordan & Evermann (D. XII, 14 or 15; A. III, 13). The second fish only was measured and found to be 12 inches long over all, 9.75 to base of caudal, 4.75 in depth. The classification was for a time in doubt, though the relative measurements fitted *D. holbrooki* quite closely. The general body-color was, above grayish faintly tinged with yellow, below silvery. Above the lateral line in specimen No. 1 were 7 narrow longitudinal stripes, below 14. Running obliquely across these were 4 or 5 dark bands. On the second specimen were 8 dark bars above the lateral line and 15 below, but the vertical crossbars were not visible.

In both, the incisor teeth were broad and stood obliquely in the jaws. The profile was very steep, and at the anterior angle of the eye was a slight convexity or protuberance. The dorsal spines were heteracanthus—*i. e.*, set staggered—so that when depressed they fitted easily into their sheath or groove. The pectoral fins reached nearly to the origin of the anal and the pelvics beyond the origin of the anal—markedly beyond in the second specimen. This particular fish had the hinder edge of the operculum black and had on the caudal peduncle a black saddle located on the dorsal and lateral parts only, as shown in Jordan & Evermann's figure 555*a* for the young of *D. holbrookii*.

The scales count of No. 1 was 8-62-16=86, with a possible error of 2 scales. In the second the vertical count was 8-9, the lateral 66-68. The pelvic fins of this specimen had accessory scales nearly as long as the inner rays of the fin.

This fish had been recorded from the Tortugas by Jordan & Thompson on the authority of Dr. Mayor but they had no specimens.

Often during the seasons of 1912 and 1913 I saw numbers of a spotted-tailed pin fish on a small reef and under the dock on the eastern side of the Tortugas laboratory on Loggerhead Key. These were thought to be *D. holbrookii,* with which I had been well acquainted at Beaufort, N. C. None, however, had been taken by hook nor in the traps, nor had any been speared. In fact no particular effort had been made to capture the fish. However, late in June 1913 some were taken in a trap off the western dock and several were grained, and all were identified as *D. Holbrooki.*

XVI. Family Gerridæ. The Mojarras.

A small family of carnivorous tropical fishes sharply marked off by having mouths protrusible, and descending when protruded, and the second interhæmal spine generally modified from the normal. Four species are found at the Tortugas, but I have examined only one.

44. *Xystæma cinereum* (Walbaum). Mojarra, "Shad." Jordan & Evermann, II, 1898, pp. 1372-1373, pl. ccxviii, fig. 556. Evermann & Marsh, 1900, pp. 207-208, fig. 61; Jordan & Thompson, 1905, p. 243.

My solitary specimen, a female, was taken in a net on the east side of Loggerhead Key. Its extreme length was 13 inches; to the base of the caudal 11; depth 4; spread of caudal 4.75; diameter of eye 0.9 inch.

This fish, which is the sole species of its genus, possessed very marked characters. The mouth had very thick lips, which were extraordinarily protrusible (1.25 inches in this specimen) downward, giving the fish a downward-hanging mouth, resembling somewhat that of certain African mormyrids. The eye was enormously large—nearly an inch in diameter in this specimen. The dorsal and anal fins—both spinous and soft parts—were depressible in a prominent scaly sheath. The caudal was deeply forked, almost to the scaly base, into equal lobes having a wide spread—4.75 inches in this 11-inch specimen. The pelvics had a very large, lobe-like accessory scale. Body-color bluish-ashy with 8 dark bars on each side extending from the base of the dorsal to the lateral line and below it for about an equal distance, where they disappeared in the light-colored, lower lateral region. Evermann & Marsh say "about 6 bars," Jordan & Evermann say 7 or 8, and their figure (which is identical) shows 8, which is the correct number, judging by my specimen.

Internally the structures were rather interesting. Pyloric cœca were absent. The prominent spleen was about the size, shape and color of a black kidney bean. Evermann & Marsh say that this fish is carnivorous. The stomach of my specimen contained about an inch of some large polychæt worm; the contents of its intestine could not be determined. It seems probable that it is a bottom feeder, the down-turned mouth fitting it admirably for seizing tubicolous worms when they come out to feed. Abdomen with paired post-anal extensions to receive the fine pointed horns of the bifurcated air-bladder. The second interhæmal was dagger-shaped, not spoon-shaped, there being no connection with the air-bladder.

Wm. Lee Wilson, of Nassau, Bahamas, tells me that this fish is called "shad" in the Bahamas, where it abounds in salt-water creeks and lagoons. In this connection, it is interesting to note that it was called "shad" by Catesby, who first described it in 1731 from the Bahamas. Jordan & Thompson quote Tortugas fishermen as calling it "narrow shad."

XVII. Family Kyphosidæ. The Rudder Fishes.

The rudder fishes are generally speaking herbivorous, having incisor teeth in the front of the jaws, wherewith to nibble at the algæ and corallines which constitute their food. In correlation with their food, they have elongated intestinal tracts. Two species were studied at Tortugas.

45. *Kyphosus incisor* (Cuvier & Valenciennes). Rudder Fish. Jordan & Evermann, II, 1898, p. 1386; Evermann & Marsh, 1900, p. 211; Jordan & Thompson, 1905, p. 243.

Only one specimen of this fish was taken. Its extreme length was 12 inches; to base of caudal 10; depth 4 inches. The lateral line scales were 11-66 (10-65 according to the authorities). No "pores 20" were found. The head was 5 in the extreme length, 4.5 in length to base of caudal; depth 3 in extreme length, 2.5 in standard length; anal 1.25 times higher than the soft dorsal. The pectorals had each an accessory scale. The pelvics had a median accessory scale. There was no depression between the spinous and soft dorsals.

The fish was lead-colored. Above the lateral line were 8 stripes of faint yellow and blue alternating with each other; below the lateral line were 16 of these. The blue stripes in the mid-lateral region were twice as wide as the brassy or yellowish ones separating them. The other matters of color agreed well with Evermann & Marsh.

46. *Kyphosus sectatrix* (Linnæus). Jordan & Evermann, II, 1898, pp. 1387-1388, pl. ccxix, fig. 559; Evermann & Marsh, 1900, pp. 211-212, fig. 63; Jordan & Thompson, 1905, p. 243.

Numbers were seen but notes were made for only one. Measurements for this specimen were: extreme length 10.5 inches; to base of caudal 8.5; depth 3.9 inches. Through some oversight no count was made of fin rays. Evermann & Marsh agree with Jordan & Evermann that the dorsal has XI spines, but their figure (copied from J. & E.) has XII. The soft rays could not be made out in the figure because of the scales with which they were thickly covered.

Third anal spine longest as shown in their figure, not the second, as stated in both references. Lobes of caudal equal as shown in figure, not lower lobe longer as stated by both. Dorsal spines placed staggered in a low sheath. Longest dorsal spine 4 (not 5) in depth. In each *jaw* 35 to 40 teeth, not in each *side* as both sets of authorities state. Color about as stated by the authorities, but my specimen had about 16 stripes or bands below the lateral line and 9 above, due to the fact that the edges of the scales were slightly yellowish, and where these scales intersected they made diffuse stripes. These are faintly indicated in the figure referred to, but not counted in the descriptions.

The internal organs were of even greater interest than the external structures. At the junction of the œsophagus and stomach was a large pouch-like diverticulum to the left, which communicated with both œsophagus and stomach by a common opening. Since this fish is herbivorous, it may be conjectured that this pouch is for storage purposes. Just beyond the common opening referred to, the alimentary canal widened out into a true stomach, at the pyloric opening of which (below or behind) a considerable number of vermiform pyloric cœca were given off. For these structures (cœca omitted) see Plate III, figure 1. The liver lay ventrad to and embraced the stomach. The much-coiled intestine was 40 inches long in this

fish (unfortunately the length of the abdominal cavity was not measured). The height of this cavity was very great in front, where it extended from the abdominal wall clear to the backbone; at the vent, however, it was very shallow—hardly more than one-fifth as high as it was anteriorly. To make up for this, however, a pair of post-anal extensions of the abdomen reached back as far as the sixth soft anal root, a distance of 1.75 inches—the roots of these rays being connected by a thin transparent membrane. This abdominal cavity, minus the extensions, is shown in Plate III, figure 2. The air-bladder was greatly elongated with a single horn extending forward above the heart clear to the base of the skull. Behind, it was bifurcated, the 2 horns completely filling the paired post-anal extensions of the abdominal cavity. The air-bladder was very thin—almost translucent. The peritoneum was golden-bronze in color underlain with black.

This herbivorous fish was provided with incisors for biting off the algæ on which it feeds, and in addition to the teeth on jaws, vomer, palatines and tongue, it had very fine teeth on both supra- and infrapharyngeals.

The generic name has no significance for the reader; however, the specific term, derived from *sectator,* one who follows, is significant, as is the colloquial name rudder fish. It is known to follow in the wake of vessels, probably to feed on waste from the galleys.

XVIII. Family Pomacentridæ. The Demoiselles.

This large family of fishes is strictly tropical. Its members are omnivorous, being nibblers and browsers on the algæ, corallines and corals with which the reefs abound. They are brightly colored and very active fish. Three species were studied.

47. *Eupomacentrus fuscus* (Cuvier & Valenciennes). Brown Cockeye Pilot. Jordan & Evermann, II, 1898, p. 1552; Evermann & Marsh, 1900, p. 224, pl. 27; Jordan & Thompson, 1905, p. 244.

Found everywhere about coral heads and loose rocks. A very saucy and inquisitive little fish. On one occasion, while in bathing at the eastern dock, an *E. fuscus* was noticed swimming about some coral blocks piled around the foundations of the posts and taking an occasional nibble at something edible attached to these rocks. On extending my bare foot to it, it left the rock and with delightful fearlessness began to nibble at one of my toes.

The description and figure of *E. fuscus* given in the Porto Rican fishes do not agree very well with specimens taken at the Tortugas. Evermann & Marsh provisionally distinguish 3 species of *Eupomacentrus* from Porto Rican waters, viz., *E. fuscus, E. analis* and *E. leucostictus.* However, as the diagnoses depend largely on differences of color, they very cautiously say that they are not sure but that all are variants of one species, *E. fuscus.* Jordan & Thompson likewise found great variation in the colors of their specimens. In addition to the 3 species above they found *planifrons,* which has not been seen by me.

48. *Eupomacentrus leucostictus* (Müller & Troschel). Beau Gregory, Cockeye Pilot, Black Pilot. Jordan & Evermann, II, 1898, pp. 1555-1556; Evermann & Marsh, 1900, pp. 226-227, pl. 28; Jordan & Thompson, 1905, p. 245.

Like its close relative, *E. fuscus,* the Beau Gregory is found very abundantly about rocks and in places affording shelter. It is a far prettier and

more attractive fish than the other cockeye pilot, but has the same qualities of inquisitiveness and fearlessness. I have seen it regale itself on the succulent crawfish meat used as bait in a trap, the meshes of which were sufficiently large to permit its comings and goings. While in bathing one morning on the eastern side of Loggerhead Key, one swam up to me and calmly nibbled at my fingers.

Evermann & Marsh's figure has the lateral line extending to a point under the middle of the soft dorsal fin, but their description says "ceasing under middle dorsal rays." All specimens examined by me agreed with the figure. Their figure does not show the marked serrations on the preopercle, though these are quite distinct. Also, the figure has too few blue spots on the soft dorsal and on the body below the lateral line.

49. *Abudefduf saxatilis* (Linnæus). Cockeye Pilot, Pintado, Sergeant Major. Jordan & Evermann, II, 1898, p. 1561; Evermann & Marsh, 1900, pp. 227-228, fig. 64.

Color while alive, brassy or lemon-yellow above, below on sides bluish, a tinge of green about the lateral line where the two colors meet. After death the sides take on a bluish tinge below. Sides with 5 vertical dark bars, velvety-black in life, purplish in dead specimens. A sixth bar, at the root of the caudal, not visible in the dead specimen, but plain on 2 live adults (5.5 and 6 inches long), and on a 1-inch young one in an aquarium there was a trace visible. These bars hardly extend down to the region of the belly.

A 4.75-inch specimen was noticed to have its left operculum distorted and greatly enlarged. Dissection showed this to be due to the presence of a crustacean parasite which had fastened itself in the gill-chamber just under the operculum. It had evidently attached itself when it and the fish were both young and had grown up *in situ,* for not only was the operculum much distorted but it was greatly hypertrophied, due to an enlargement of the opercular bones. In corresponding planes the left operculum was from 3 to 5 mm. wider than the right. Such cases of parasitism are common, but this is the most exaggerated the writer has ever seen, the little fish looking as if it had a bad case of mumps on the left side.

This saucy and interesting little fish is found everywhere among the coral heads associated with the other cockeye pilots of the genus *Eupomacentrus.* It has a very knowing way of dodging in and out of the interstices of corals and between the fronds of the corallines and is very hard to catch save in traps.

50. *Microspathodon chrysurus* (Cuvier & Valenciennes). Jordan & Evermann, II, 1898, p. 1567, pl. ccxxxv, fig. 593; Jordan, 1904, p. 540; Jordan & Thompson, 1905, p. 246.

There were studied 2 specimens taken with dynamite in coral heads on the west side of Loggerhead Key. These measured: No. 1, length over all 5.75 inches, to base of caudal 4.5, depth 2.5 inches; No. 2, length to tip of caudal 4.75 inches, to base of caudal 4.25, head only 1.4, diameter of eye 0.4, depth 2.5 inches. The fin formulas for both were D. XII, 15; A. II, 13. Jordan & Evermann say D. XI, 15; A. II, 13, but their figure has D. XI, 15; A. II, 12. However, they must be in error here; both my specimens had D. XII, 15, and all the other Microspathodons are noted in Jordan & Evermann as having 12 dorsal spines. The number of anal rays in the various species runs 13 to 14, sometimes varying in the species *(niveatus),* but their text says for this species 13 and their figure shows 12.

The lower jaw was squarish, and both jaws were provided with movable teeth suitable for eating algæ. An oral breathing valve was present above. The mouth was somewhat protrusible. Both the whole head and the opercular region were densely scaled, as were the basal halves of the dorsal and anal fins. Pelvic fins with marked accessory scales bent longitudinally to form a right angle. Upper lobe of caudal fin longer than lower, not equal as shown in Jordan & Evermann's figure. Lower edge of preopercle more or less serrate. Lateral line extending from back of eye to the notch made by hinder edge of soft dorsal with upper part of caudal peduncle, not stopping short of it as shown in the figure referred to. Blue spots very distinct between eyes, on nape, on both spinous and soft dorsals, and between these and the lateral line.

Jordan & Thompson report this fish as not unusual at the Tortugas; only 2, however, were taken at the laboratory during my visits.

XIX. Family Labridæ. The Wrasses.

The Wrasses comprise a very large family of tropical fishes, embracing 60 genera and nearly 500 species. Among their characteristic structures are the protrusible maxillaries provided in front with strong canine teeth for crushing the mollusks on which they feed. Characteristic also is the T- or Y-shaped single lower pharyngeal formed by the ankylosis of the paired bones. Most of the Labrids are brilliantly colored and many are valued for food. The various species seem subject to much variation.

51. *Lachnolaimus maximus* (Walbaum). Hog Fish, Capitan. Jordan & Evermann, II, 1898, pp. 1579-1580, pl. ccxxxvii, fig. 597; Evermann & Marsh, 1900, pp. 230-231, fig. 65; Jordan & Thompson, 1905, p. 246.

This fish was not uncommonly seen around coral heads. Four specimens were examined and 3 measured; 2 of these were small, 11 and 11.5 inches in extreme length, and 3.9 and 4 inches deep respectively. The largest was 19.75 inches outside length, 6.75 inches deep and weighed 4 pounds. The second dorsal filament was longest (6 inches), reaching to the beginning of the soft dorsal, which had 12 rays instead of 11 as called for by Evermann & Marsh, but agreeing with their figure which was copied from Jordan & Evermann, who allowed 11 or 12 soft rays for the dorsal. The mouth had 4 large canines above and 2 below, and was extraordinarily protrusible—the vertical gape in this specimen, when the mouth was fully protruded, being 5 inches.

On this large specimen, 3 short blue stripes were noted under the eye. In the 11.5-inch specimen, the iris was red, the pupil when looked at squarely was black, but when viewed obliquely a bronzy reddish-green. The pectorals were a pale lemon-yellow; the ventrals darkish, purple at the tip. The soft dorsal had a black spot at the base. The tip of the anal was very long, overlapping the first third of the lower lobe of the caudal. This specimen had had the tip of the lower lobe of the caudal bitten off.

A fourth specimen had in its intestinal tract fecal débris mixed with a considerable amount of coral fragments, for the nipping off of which its strong frontal canines are admirably suited. The large gape, however, would seem to indicate that it swallows crustaceans and fishes of considerable size whole.

This fish, with its brilliant coloring (of which it has 3 or 4 phases), its unusual figure, its elongate three anterior dorsal filaments, its pointed soft

dorsal and anal, and its extreme lunate caudal due to the marked elongation of the outer upper and lower rays, is one of the most showy fishes at Tortugas. It grows to considerable size, reaching a weight of 12 or 15 and sometimes 18 or 20 pounds. It is an excellent food fish. The local name, hog fish, is probably given on account of the shape of the snout.

52. *Iridio bivittatus* (Bloch). Slippery Dick, Doncella. Jordan & Evermann, II, 1898, pp. 1595-1596, pl. ccxxxix, fig. 601; Evermann & Marsh, 1900, p. 232, fig. 66; Jordan & Thompson, 1905, p. 246.

Probably the most abundant fishes around the coral heads in the Tortugas are the Iridios. Likewise they are probably the most beautifully colored. Jordan & Thompson make 3 species of these, the differences being largely based on the coloration.

The Slippery Dick swims rather slowly, with a sinuous eel-like motion of the hinder part of its body mainly lateral but sometimes vertical. Further, because of the smallness and weakness of its thoracically placed pelvic fins, it has great difficulty in preserving an upright position when at rest on the bottom. In an aquarium it was often noticed to take advantage of any inequalities to lean up against these in the endeavor to preserve its equilibrium. If the bottom were perfectly smooth, the fish would often lie on its side—one such was thought to be dead but when touched swam away—but more often the fish would assume a sinuous flat S-shaped curve, and by this means keep itself upright as shown in Plate III, figure 3. When some sargassum weed was put in an aquarium with a number of Iridios, they very promptly betook themselves to it and, as it were, lodged themselves in it. One was noticed which so perfectly adapted its colors to that of the weed as very effectually to conceal itself. During the four summers that these fish were kept in aquaria at Tortugas not one was ever seen to close its mouth in breathing. The regurgitation of the inspired water was prevented by oral breathing valves, the one below being very prominent.

The name *Iridio*, from *Iris*, the rainbow, is certainly well applied; the specific term *bivittatus*, two-banded, refers to the two dark longitudinal bands on the sides. And any one who has ever tried to hold one of these fish in his naked hand can testify to the soundness of the colloquial appellation Slippery Dick. Tortugas specimens are small, ranging from 3.5 to 5 inches, other species of this genus, inhabiting tropical waters, often exceed a foot in length.

XX. Family Scaridæ. The Parrot Fishes.

The Scaridæ are brilliantly colored, herbivorous, tropical fishes, often rather large. They are not esteemed for food, the flesh being soft and not very good. They are notable for their coalesced teeth which form jaws or beaks (hence their colloquial name), and for their unusually large cycloid scales.

53. *Sparisoma abildgaardi* (Bloch). Red Parrot Fish. Jordan & Evermann, II, 1898, p. 1635; Evermann & Marsh, 1900, p. 239, fig. 30; Jordan & Thompson, 1905, p. 247.

One specimen was examined, which measured: length over all 11 inches; to base of caudal 9.25; head only 2.63; snout 1.25; depth 3.75; diameter of eye 0.5 inch. The fin-ray count was D. IX, 10; A. II, 9. This agrees with Evermann & Marsh's count but not with their colored figure, in which their artist has put 8 spinous and 11 soft dorsal rays. The above specimen was

taken during my absence from Tortugas and had been in formalin for 24 hours when examined. My notes simply say that the colored figure is not well done and does not correspond with the colors of the formalin specimen.

Between the pelvic fins were 3 interesting overlapping scales as shown in the subjoined pen sketch, Plate III, figure 4.

In the parrot fishes the lateral line and the scales bearing it are of especial interest. In *S. abildgaardi,* behind the eye the lateral line was spread over 3 scales of the preopercle and the opercle. Behind these it covered 4 scales ranged in an antero-posterior, dorsi-ventral (oblique) direction. Thence it ran straight back to scale 17, where it dropped down one row and spread over 2 rows of scales, a distance of 4 scales backward. On scale 21 it again became single and so extended backward to the base of the caudal. In other words, on the head parts there is a plexus and another at the hinder edge of the base of the soft dorsal. Each scale has the lateral line in the form of a tree having a short trunk, which breaks up into a great number of branches—a deliquescent tree as shown in the sketch, Plate III, figure 5. Only in the extreme posterior region do the scales have this arborescent structure in the form of an excurrent tree, *i. e.,* having a distinct trunk from base to tip.

54. *Sparisoma viride* (Bonnaterre). Dark-green Parrot Fish. Jordan & Evermann, II, 1898, p. 1638; Evermann & Marsh, 1905, p. 240; Jordan & Thompson, 1905, p. 247.

The one specimen (examined by me, after it had been in formalin 2½ days) was taken with dynamite off the northwest side of Loggerhead Key. Its measurements were: length to tip of caudal 10.75 inches; to base of caudal 9; of head 2.75; diameter of eye 0.44; depth 3.75 inches. Since it had been in formalin no notes of its color were made save that it agreed pretty closely with Evermann & Marsh's description.

Much trouble was experienced in classifying this fish, using the *Fishes of Porto Rico.* The key says "caudal deeply forked," the description says "caudal deeply lunate," which is correct. The key says "opercle with inky-black spot, in front of which is a golden spot"; whereas the description says that the operculum has a small pale lemon spot, and below it a green one—and this is correct. Jordan & Evermann, whose key Evermann & Marsh copy, say in their description "no inky-black spot on opercle."

In *S. viride,* as in the preceding *S. abildgaardi,* the lateral line is of particular interest. It was spread over the whole opercular region back of the eye. Behind this it covered five scales in two vertical rows, then three scales, next it trailed off backward in 16 single scales, making 19 from the beginning backward. This was the situation on the left side. On the right the anterior arrangement was the same but there were only 14 single scales, making only 17 from the beginning. The last lateral line scale on each side was long and pointed, with an excurrent tree-like figure made by the lateral line tubules, as shown somewhat diagrammatically in figure 6, Plate III.

55. *Sparisoma frondosum* (Cuvier). Parrot Fish. Jordan & Evermann, II, 1898, p. 1642.

My one specimen was grained in shallow water at the northeast end of Long Key. It measured 15 inches to tip and 13 to base of caudal, and was 4.5 inches deep. The scale count was 2-25-5. The fin rays were D. IX, 10; A. II, 10. The eye was contained 6 in the head. The coloration was not

a "uniform dark purplish violet" but a uniform brownish olive on body and fins. However, the colors of these fish are so liable to change with the changing emotions of the fish, and since this fish had been taken with the grains (a second stroke being necessary), this may merely be one of its color phases.

Except for the color this fish agreed well with the brief description given by Jordan & Evermann. It was so cut up with the grains that but few notes were made of it. The pelvic fins, which were inserted under the pectorals, were provided with accessory scales of large size; and the pectorals themselves had between their bases a single large accessory scale. All the scales were very large; those in the lateral line being covered with the much branched tubules—whence the name *frondosum*. The mouth was provided with breathing valves above and below. This specimen was a female with immature eggs.

This fish was taken in a little bay at the northeast end of Long Key, in water so shallow that it was lying somewhat on one side (its body inclined at an angle of 45°) against a piece of dead coral rock. When I first saw it it had attached to it a small *Echeneis*, but when the grains failed to hold in the large thick scales at the first stroke, this little fish got away in the commotion and could not be found.

This fish has not heretofore been reported from the Tortugas, and it is also new to the waters of the United States.

56. *Scarus cæruleus* (Bloch). Blue Parrot Fish. Jordan & Evermann, II, 1898, pp. 1652-1654; Evermann & Marsh, 1900, p. 244, pl. 32.

Five specimens were examined: 10.5, 10.75, 11, 11.75 inches in extreme length. Full measurements were made for the fifth fish: length to tip of caudal 11.5 inches; to base of caudal 10.25; of head 3; diameter of eye 0.44; depth 4 inches. The fin-ray count was normal in every specimen: D. IX, 10; A. II, 9. In the plate referred to above, the colored figure has the spines correctly shown, but both dorsal and anal appear to have an extra soft ray.

The first 4 specimens were taken alive in traps and careful notes were made of their color as follows: iris golden; upper edge of eye deep blue; lower lip pale blue, under this a flesh-colored (not orange) band 4 times wider than the blue band; upper part of head olive-green; the dorsal fin olive-green; fin rays faintly blue; a longitudinal stripe of deep blue on fin ray IX; bases of paired fins deep blue; rays of anal fin a darker blue than those of dorsal. These colors were identical for 4 fish. Later one of these, in an aquarium, some hours before death, showed 4 irregular flesh-colored bands across the body. Comparison of these notes with the colored figure of the Porto Rican fish will show marked differences of color, and further comparison of the different descriptions indicate that the fish has a number of color phases. However, the predominant color is blue, and hence the specific name.

The fifth specimen was taken with dynamite during my absence and had been in formalin 3 days when examined, hence no notes on its color were made. However, it differed structurally in several points from the fish figured in the colored plate. The snout was blunt and rounded but did not show so much overhang as that figured. The forehead between the eyes was slightly convex, not concave. An inferior oral breathing valve was noted. The pores and canals of the lateral line were not doubled nor branched as shown in the figure. The lateral line began in a curve starting just above the eye

and extended straight back to the eighteenth scale, where there was such a plexus as shown for both right and left sides in Plate III, figure 7. The last scale only on each side showed a somewhat branched lateral line. All the scales were very large.

XXI. Family Chætodontidæ. The Butterfly Fishes.

The Chætodons are omnivorous tropical fishes, noted for their bright colors, unusual shapes and for their restless activity. They form charming pictures as they play in and out of the corals and seaweeds on the reef.

57. *Chætodon ocellatus* (Bloch). Butterfly Fish. Jordan & Evermann, II, 1898, p. 1674, pl. CCXLIX, fig. 621; Jordan & Thompson, 1905, p. 248.

Of this striking little fish I have had 3 specimens and have seen scores around the reefs. Two only of these 3 had the dorsal rays counted. These were XII-21 and XIII-19. In both specimens the anal rays were III-17. The second fish only was measured: length over all 4.25 inches; to base of caudal 3.6; of head 1.25; depth 2.5; eye diameter 0.37 inches.

The life colors of 2 specimens were noted. The ground color of each was cream-white becoming slightly grayish on the tail. The spinous dorsal was deep lemon-yellow, the soft dorsal a paler yellow. At the central base of this latter was an obscure dark spot and its tip was deep black behind. The base of the caudal and major portion of the fin were also a deep lemon-yellow. The tip of this fin was edged with a white band 2 to 3 mm. wide. In front of this was a yellow band of about the same width, and anterior to this another very narrow white band, then came the deep yellow noted above. The anal was a deep yellowish-orange tipped with a bright yellow band 2 mm. wide, preceded by a 1-mm. white band—as on the tip of the caudal. The pelvic fins and the bases of the pectorals were of the same bright yellow, as were the hinder edge of the opercle and the neighboring parts of the body. The preopercle and upper snout were a lighter yellow. The black ocular bands edged with yellow accurately bisected the eye through the pupil as shown in Jordan & Evermann's figure. Between the eyes and across the forehead, a faint yellow bar transverse to the ocular bands made an H-shaped figure, the regions above and below the crossbar being of a brighter yellow.

My second specimen, whose colors were noted, was taken with dynamite on Loggerhead Reef. These colors some hours after death differed from the live specimens, mainly in intensity, being paler and less marked, approaching somewhat those recorded by Jordan & Evermann. This was especially so of the soft dorsal fin, its colors closely approaching those in their figure.

The figure and the description by Jordan & Evermann do not tally in all points—for instance the latter calls for a black spot on the upper opercle, and this is lacking in the figure as it was in my specimens. Both figure and description seem to have been made from dead and probably alcoholic specimens. The figure shows a dark band across the side from the soft dorsal to the similar part of the anal. This was noted only on my dead specimen. If comparison is made between description and figure of *C. ocellatus* in Jordan & Evermann and *C. striatus* in Evermann & Marsh, the imperfections of the former will be seen at once.

Thompson in Jordan & Thompson (1905) notes the color very much as it has been given above. He says that the black band across the body from dorsal to ventral is "sometimes" found or that the sides may have a mottled

appearance. In this connection it may be noted that the specific term *ocellatus* seems to be a misnomer when applied to this fish, as it does not have any eye-like spot.

The fish presents a striking appearance as it swims in and out of the coral rocks, especially as it has the habit of leaning over on its outer side as it turns corners, thereby giving an excellent view of its striking coloration.

58. *Pomacanthus arcuatus* (Linnæus). Black Angel Fish. Jordan & Evermann, II, 1898, pp. 1679-1680, pl. CCLI, fig. 623; Evermann & Marsh, 1900, p. 251, fig. 69; Jordan & Thompson, 1905, p. 248.

Numerous specimens of this common fish were measured and examined. Some measurements (in inches) are as follows:

No. of specimen	Length to tip of caudal	Length to base of caudal	Depth	Spread of dorsal and anal fins	Weight in pounds
1	9.75	.....	6.0	.....	0.5
2	14.0	.....	8.25	13.5	2.5
3	13.5	.....	8.0	13.0	2.0
4	10.75	.....	6.25	9.0	.....
5	18.75	15.75	10.5	16.0	6.5
6	13.5	11.75	9.5	.....	.....

The fin-ray count was D. IX, 31 or 32; A. III, 23; agreeing well with the authorities. However, their count D. VIII or IX, 30 to 32; A. III, 24, is not borne out by their figures, which are made from the same block. On it, 22 soft rays can be made out in the anal, but only 19 to 20 in the soft dorsal. No especial trouble was had in counting them in the Tortugas specimens.

The mouth was protrusible to the extent of an inch in my largest specimen, due to the forward extrusion of the upper jaw which had attached to it, at its middle-point, a freely moving bone, presumably the posterior fused processes of the premaxillæ. Mouth when protruded gaped widely—in my largest specimen 0.75 inch vertically by 1.1 from right to left. Teeth in rows in front part of mouth forming a kind of pad (in a 10.75-inch specimen this was 3 mm. wide). The rows, however, seem to be broken into segments having 5 to 8 teeth on each. Teeth long and slender, hinged at the base to move right and left but not front and back. The middle lower teeth in specimen No. 4 were 5 mm. long and 0.5 mm. thick. They were pointed at the tip with 2 lateral, sharp cusps about half as high as the main point, as shown in figure 8 of Plate III.

The digestive tract was long and coiled. Stomach thick walled and densely rugose—thickest at pyloric end. Fish No. 3 (13.5 inches long) was carefully dissected and its alimentary canal measured. Œsophagus and stomach measured 6 inches, small intestine 58 inches, large one 21 inches; total 85 inches, over 7 feet not counting the 21 cœca. All this was packed away in an abdominal cavity whose greatest measurements were 5 inches long, 1.5 wide, 3.25 deep; and which contained in addition the air-bladder and the slender paired immature ovaries. The largest fish (18.75 inches) had an alimentary canal (mouth and œsophagus not counted) 75 inches long. This was emptied and found to contain 240 c.c. of crustacean remains, pieces of gorgonians and fragments of algæ of various kinds. All this was contained in an abdomen which measured 6.25 inches long, 4 deep and 1.5 wide, and of which the air-bladder occupied one-third to one-half.

The fish is plainly an omnivorous feeder, and not carnivorous as commonly thought. I am inclined to think that its principal food is algæ. The intestine is extraordinarily long, the contents under the microscope show much algal matter.

The abdomen had a paired post-anal extension on each side of the roots of the dorsal rays. In the 10.75-inch fish these extensions were 2 inches long. They were occupied solely by the posterior horns of the air-bladder.

The pectorals (which were slightly more than half as long as the pelvics) were, on the front side, dark at the base, transparent at the tip and outer edge and all the remainder bright yellow; behind this the fin was yellow. Against the body this fin appeared black, but when held to the light it was yellow. Lower jaw white, upper blue or dark in front, white behind in the groove. Nostrils rimmed with bright yellow. In one fish there were no spots on the preopercle as shown in the figure referred to (E. & M.), but the preopercle and spine were much larger than figured, the spine reaching clear to base of pectoral fin.

The scales of the black angel fish are of two sizes, large and small. The large scales are transparent and, under each is a little ear- or scale-shaped lappet of skin, containing a black spot which makes the scale look black. When the scales are removed the spots appear much blacker. The coloration under the scales and the arrangement of the scales give the fish roughly the appearance of having faint vertical bands. The hinder edges of the soft dorsal and anal fins, particularly the filaments, were a dark almost indigo-blue, in front of this was a sage-green velvety region in one fish. Caudal generally tipped with a flesh-colored band (one had here a pale blue band), anterior to this a band of faint yellow about half as wide, in front of this a dark region grading into the gray of the caudal peduncle.

Among miscellaneous data worth recording, it may be noted that the fish swimming in an aquarium kept their mouths open and protruded, with the upper oral breathing valve going rapidly. The dorsal filament was noted as generally about 1.5 times the length of the anal filament, and not shorter, as the figures referred to have it (they being identical). In my largest specimen the vertical spread of the dorsal and anal filament was 16 inches. The fish when turning a corner lies down on its outer side as noted for other broad fish. Last of all the following differences between my specimens and the figure and descriptions may be set down. In mine the dorsal and anal filaments extended beyond the tip of the caudal (one fish had 2 anal filaments) and were much more slender than those figured (see Plate III, figure 9); the lateral line was more arched and more distinct, ending on the caudal peduncle rather above the mid-lateral region; the spine of the preopercle was much longer and the preopercle was not spotted; the rays of the dorsal and anal fins were much more apparent; there was present a deep groove between the eye and the nostril; no white line extended from the nape across the head.

59. *Angelichthys ciliaris* (Linnæus). Blue Angel Fish, Isabelita. Jordan & Evermann, II, 1898, pp. 1684-1685, pl. CCLIV, figs. 626, 626*a*; Evermann & Marsh, 1900, pp. 252-253, pl. 37; Jordan & Thompson, 1905, p. 248.

Of this quite abundant fish but one specimen was worked over. Its length between perpendiculars was 14.5 inches; to base of caudal 12.5 inches; to tip of dorsal streamer 17.5 inches; depth 6.5 inches. It weighed 2 pounds.

The body-color was a bright blue, as were the fins at their bases. Pectoral, pelvic and caudal fins were bright yellow and the tips of dorsal and ventral ended in a bright lemon-yellow. All the spines, both of dorsal and ventral fins, were a bright blue verging on to purple. The colored plate (No. 37) in *Porto Rican Fishes* is pale and washed out compared to the living fish as seen swimming in and out of the coral rocks at Tortugas. Preorbital with 4 spines in my specimen; 3 are referred to by Evermann & Marsh, but none figured in plate 37. The 9 longest preopercular spines were each about half as long as the large basal spine, which in this specimen was 0.5 inch long. All these spines were a bright blue.

XXII. Family Teuthididæ. The Surgeon Fishes.

These short, deep, steep-headed fishes are definitely set apart from even their nearest neighbors (the Balistidæ) by the presence on each side of the caudal peduncle of a bony lancet lying in a groove. This is free at its forward end, but so provided with muscles attached to the basal and posterior end, as to erect it so that it will point forward, making an angle of about 45°. With this the fish can inflict a sharp wound, whence its trivial name. It would be an excellent, even if a small, morphological problem to work out the muscles and nerves supplying this spine.

60. *Teuthis cœruleus* (Bloch & Schneider). Blue Tang, Surgeon Fish, Doctor Fish. Jordan & Evermann, II, 1898, p. 1691; Evermann & Marsh, 1900, pp. 253-254, pl. 38; Jordan & Thompson, 1905, p. 248.

One specimen (not measured) had fin rays: D. IX, 23; A. III, 21. Evermann & Marsh counted: D. IX, 26; A. III, 25. In their figure the spines are all right, the soft dorsal apparently has 26 rays, but it is impossible to make out the soft anal rays.

While this fish was alive in the aquarium no longitudinal streaks could be found, and it was thought to be *bahianus*. However, after death, on the bronzy brown-olive sides of the body, longitudinal streaks could be seen, but as these were brassy, it was still thought to be *bahianus*. Nevertheless, note was made that the parallel stripings in the dorsal fin, instead of running side by side to the hinder edge, as in *bahianus*, began to emerge about the thirteenth to fifteenth soft dorsal ray. This looked like *cœruleus*, but the fish in its general coloration did not resemble plate 38 (E. & M.). To add to the confusion, Evermann & Marsh's description and Baldwin's colored plate do not at all agree, and Jordan & Evermann agree with neither. Furthermore my one specimen had been kept in an aquarium for some time, where, refusing to eat, it had battered its head and worn its caudal fin in trying to escape. In this uncertainty the specimen was put in a tank of formalin with other fishes and later sent to the United States National Museum. Still later, when Mr. B. A. Bean came to look over this small collection, he at once recognized it as *cœruleus*. The word means blue, which it certainly is not in Baldwin's drawing; however, this species, like *bahianus*, undoubtedly has a number of color phases.

61. *Teuthis hepatus* Linnæus. Tang, Lancet Fish, Doctor Fish, Surgeon Fish. Jordan & Evermann, II, 1898, pp. 1691-1692; Evermann & Marsh, 1900, p. 254; Jordan & Thompson, 1905, p. 248.

My one specimen was studied alive in the aquarium, and also immediately after death. The fin rays were D. IX, 25; A. III, 24, agreeing well

with the authorities—Evermann & Marsh give D. IX, 24-26; A. III, 22-24; their predecessors D. IX, 26; A. III, 24.

The teeth were incisor-like, multi-cusped or pointed, in a single row attached by the base to a membrane in such a manner that they could be easily turned to right or left or bent backward. These teeth are admirably adapted to nibble algæ, on which the fish feeds. Behind the teeth the gums were black, tough, longitudinally rugose, seemingly used to hold the food nipped off by the teeth. Figure 10 on Plate III is a rough sketch of one of these multi-cusped teeth.

The life colors of this tang were very beautiful. The general body-color was a dark olive-green above and bluish below, crossed by 10 (not 12) dark vertical bars, which in certain phases were a very faint blue. Eyes with 7 blue bars behind separated by orange, the lowest swinging under eye to the front. An orange patch under each eye and 2 faint streaks in front. Hinder gill parts velvety blue-black, becoming merely a deep blue in the isthmus region. Edges of dorsal and anal fins, outer rays of pelvics and lancet region a royal purplish blue. Pectorals bluish black at base and on edges, upper rays orange in the outer half. Caudal lavender, rays blue-black in certain lights, upper lobe lavender-blue at tip. In the spinous dorsal, the stripes of blue and olive ran upward and backward, in the soft part they were about parallel, as in *T. bahianus*.

Immediately after the death of the fish the following colors were noted. The general body-color was dark, even over the bases of the dorsal and anal, but the vertical stripes persisted as noted by Evermann & Marsh. The lancet was framed in vivid blue. The blue bars around the eyes had largely faded, but the 7 intervening orange streaks were very apparent. In the eye, the pupil was now black and the iris orange with a narrow longitudinal blue streak above and below it, as seen in figure 11, Plate III.

This tang, which Evermann & Marsh say is the most abundant species in Porto Rico, is evidently given to much variation, and likewise must have several color phases. A young one taken at San Juan, Porto Rico, showed considerable color divergence from those previously described. Of particular interest just here is the statement that the eye was "blue and yellow."

62. *Teuthis bahianus* (Castelnau). Surgeon Fish, Lancet Fish, Doctor Fish, Ocean Tang. Jordan & Evermann, II, 1898, p. 1693; pl. CCLVI, figs. 629, 630; Evermann & Marsh, 1900, pp. 254-255, fig. 70.

Numbers of specimens were seen around coral heads, and 3 were studied carefully. Two of these measured: No. 1, 6.88 inches in extreme length, 3 deep; No. 2, 8 inches over all, 2.75 deep. These gave for fin-ray count: D. IX, 24; A. III, 22. Evermann & Marsh say D. IX, 25; A. III, 23, but their figure gives D. IX, 24; A. III, 21. Jordan and Evermann record: D. IX, 24; A. III, 22; while their figure, which is the original of the one just noted, has the same count. The disparity of the corresponding measurements listed above is a further proof of the marked variability of this fish.

The dorsal fin had at its base a distinct scaly sheath marked off from the body as a yellow (not violet) band embracing the bases of the rays, the scales of which moved forward as the fin rose, and backward as it was depressed. The fin itself was beautifully banded with 8 pale salmon lines (in one fish) or brown lines (in another) alternating with 7 dark ones, the whole fin tipped with purple. The caudal in one fish was a chlorine-yellow (not violet), in another the general color was not noted but the fin was tipped with blue. The pectoral rays were orange-colored, growing lighter

toward the tips. The outer half of the ventral was dark blue, and so for the anal, which had, however, a dusky olive base.

The caudal was somewhat unequally lobed, the upper lobe being about as much larger than the lower—of the same form and shape—as that shown in Evermann & Marsh's figure 70, and in none of the fish seen was the "upper lobe much the longer, slender and often produced into a filament in adult . . . outer rays . . . longer than head."

The lancet was 14 mm. long. The anterior half was free, but to the hinder part a powerful muscle was attached, and this by contraction caused the lancet to stand out from the body so that if driven against another body by a lashing of the fish's tail it would inflict a sharp wound. The very short posterior end of the lancet was free of muscle for about one-fifth of its whole length. These matters are shown in the semi-diagrammatic figure, No. 12 on Plate III.

These tangs show very marked color phases with their changing emotions, and the discussion of the color here entered into will be merely that necessary for the identification of the fish. One specimen had sides a light bronze or brassy, another was bluish. On the first no longitudinal brassy streaks could be detected, but on the second, under the glass, there were visible brown wavy lines extending over the upper half of the body. The eye had a dark-brown iris surrounding a deep dark-blue pupil. The dark-olive background behind the eye was marked by 7 deep-blue lines of about the size, shape and position as shown in the sketch (figure 13, Plate III). In front of the eye the double nostril was surrounded by a faint purple cloud becoming darker near the eye. The hinder edges of the opercle were a velvety blue-black in one fish, merely tipped with blue in another, and the isthmus region was inky-blue. The 14-mm. lancet and its surrounding parts were purple.

This species was first described from Bahia in Brazil. It has not been reported before from the Tortugas.

XXIII. Family Ostraciidæ. The Trunk Fishes.

The Ostraciidæ are highly specialized fishes with the vital organs inclosed in a trunk or box formed of solidly united, hexagonal bony plates. From this carapace protrude the jaws, fins and caudal peduncle, all of which possess some freedom of motion. The bones of the jaws are firmly united, and the gill-opening is reduced to a mere slit.

63. *Lactophrys triqueter* (Linnæus). Trunk Fish. Jordan & Evermann, II, 1898, pp. 1722-1723, pl. CCLXI, fig. 638; Evermann & Marsh, 1900, pp. 262-263, fig. 219; Jordan & Thompson, 1905, p. 249.

Four specimens of this interesting fish were studied, 2 of which were measured. These were each 9.5 inches long over all, 4 and 4.25 inches deep, and 3.5 and 3.25 inches wide on the ventral surface, respectively.

The mouth was made up of two protrusible lips, thick, leathery and rounded in outline, markedly similar to the lips seen in bill-board posters of negro minstrels. Upper jaw with 10 incisor-like teeth, close-set and immovable like the teeth in a "plate" of artificial make for the human mouth. Upper lip and teeth closely joined, no space between, as in the human mouth. Eight close-set incisors in lower jaw, but with some space between teeth and lip. Teeth brown with corn-colored or yellow tips.

Gums growing in irregularly shaped masses between the bases of the teeth exactly as in the human mouth (see figure 14, Plate III, for a representation). The whole mouth apparatus fitted for biting, nipping—*i. e.*, the fish is a browser, a feeder on algæ and similar growths. In breathing the mouth was protruded from the opening of the shell, and the lips were kept in rapid motion; at the same time the movable edge of the gill-cover was kept in rapid synchronous vibration. I know of no fish that breathes so rapidly—this one fairly pants for breath. No oral breathing valves could be seen, but, as the mouth is never closed, such must be present.

Pectoral, dorsal and anal fins were movable at their bases through holes in the armor. The tail protruded through a hole in the bony box behind, exactly as the mouth does anteriorly. Caudal freely movable and is *the* organ of locomotion. A broad keel along each lateral-ventral margin enables the fish to preserve its equilibrium and also to glide over stony or any unequal bottom. From experience I can certify that the trunk fish can scud along close to the bottom with considerable celerity and can turn corners so readily as to make the graining of it a matter of considerable difficulty.

The large eyes were protected by bony brows or orbits on the inner and upper region, and these extended backward as ridges below and parallel to the median ridge and finally melted away into the bony carapace. The movable eyes set in this bony armor solemnly follow one's movements and remind one of a mediæval knight staring out of the holes in his helmet.

Below the eye the nearly vertical gill-opening was guarded by its ceaselessly moving opercular flap. When brought in, the fish floated at the top of the water, probably because it had swallowed air after the fashion of the puffers. Another fish spouted water out of its mouth (again like the puffers) clear over the sides of the bucket, a distance of 6 inches. Later it stood on its tail and made a vigorous effort with its pectorals to get out of the bucket. The entrails having been drawn, this fish when cooked in the shell is said by our laboratory men to be delicious eating.

64. *Lactophrys bicaudalis* (Linnæus). Spotted Trunk Fish. Jordan & Evermann, II, 1898, p. 1723, pl. cclix, figs. 640, 640*a*, 640*b*; Evermann & Marsh, 1900, p. 264, pl. 40; Jordan & Thompson, 1905, p. 249.

My one adult specimen was grained by Capt. George Tracy in the bay off the laboratory wharf. Its total length was 9.24 inches, depth 3.5, width on the ventral surface 2.5. Being badly cut up by the grains, it had to be put into formalin without any notes being made on it. However, it is easily distinguished from all the other trunk fishes by reason of the spots which are especially notable on tail and caudal fin.

On July 17, 1912, Wm. Lee Wilson caught with a dip-net a little *Lactophrys* in a pool to the southeast of Fort Jefferson. It was 3.6 inches long (outside measurements), 1.6 deep, 1.4 inches wide on the ventral surface. Later in the day it died, and after being carefully studied was preserved in alcohol. Its fin-ray count was: D. 11, A. 11, P. 12, C. 10. Evermann & Marsh give: D. 10, A. 10, P. 12; and with this their elegant plate agrees, the caudal, however, having 12 rays. Jordan & Evermann give the same count and though no mention is made of the caudal their figure shows 10 rays in it. Since none of the other trunk fishes figured have more than 10 caudal rays, it would seem that Evermann & Marsh's figure is in error here.

Since my young specimen differed considerably from the recent description given by Evermann & Marsh, the careful notes made from it while fresh will be given. Forehead very concave, a deep concavity between eyes; hump of back nearly as marked as in *L. triqueter;* caudal peduncle small. Snout rose-colored; a deep black bar across forehead from eye to eye, higher on right eye than on left. Across dorsal ridge about halfway between front edge of eye and front edge of dorsal, a dark Λ-shaped mark, apex of the Λ forward, legs extending obliquely backward and downward; apex of Λ on dorsal ridge, legs about 4 spots wide and 6 long. At base of dorsal a very dark spot, first and second rays dark, on right side of base three large black spots, on left two. Behind pectorals a dark region extended backward and upward nearly to the Λ-shaped band, but was separated from it by a light region. Caudal with a longitudinal row of 3 black spots on rays 1 and 2, 5 on the bottom rays. Tip of caudal with a vertical row of 7 or 8 spots, anterior to this row 2 spots at the bottom edge, anterior to these a weak row of 7 vertical spots—small and rather scattered. Body everywhere light-colored with black spots mainly stellate with 6 bars, or points, as shown in detail in figure 15, Plate III. These stellate spots, set in checkerboard fashion, showed 3 lines of arrangement; nearly vertical, obliquely downward from front to back and obliquely downward from back to front. These points are brought out in Plate 4, figure 2, made from a photograph of this young specimen, the smallest (3.6 inches) ever taken so far as I know. Unfortunately, the photograph was not made until after the fish had died. Comparison of this figure with Evermann & Marsh's description will show considerable differences.

65. *Lactophrys tricornis* (Linnæus). Cow Fish. Jordan & Evermann, II, 1898, pp. 1724-1725, pl. CCLXI, fig. 639; Jordan & Thompson, 1905, p. 249; Evermann & Marsh, 1900, pp. 264-265, fig. 77.

Two specimens of this cow fish were taken in an interesting way. On June 12, 1912, a 6-foot short-nosed shark, *Hypoprion brevirostris,* was taken on a hook in the lee of Loggerhead Key. On dissecting it, 2 half-digested *Lactophrys tricornis* were found in its stomach. These were cleaned, washed, put in formalin, later dried and afterward positively identified as the above. The interior parts were gone, as was also the epidermis, leaving the shell beautifully clear and transparent, allowing its structure to be made out perfectly. For a photograph of this see Plate 4, figure 3.

XXIV. Family Gobiidæ. The Gobies.

This large family of fishes, characterized in general by the possession of a ventral sucking disk formed of the confluent pelvic fins, is represented in my collections at Tortugas by but one species.

66. *Gobius soporator* Cuvier & Valenciennes. Goby. Jordan & Evermann, III, 1898, pp. 2216-2218; Evermann & Marsh, 1900, pp. 294-295.

Numerous specimens were taken in tide-pools on the east side of Loggerhead Key, but only the largest was examined. Its fin formulas were: D. VI, 9; A. I, 9. Evermann & Marsh say D. VI, 10; A. 9. Jordan & Evermann D. VI, I, 9 or 10; A. I, 7 to 9. Its length outside to outside was 47 mm.; to base of caudal 37 mm.; its depth was 8 mm.; the head was 10.5

mm. long; and the diameter of the eye 3 mm. The head was contained in the body 3.5 times; depth in body 4.6; eye in head 3.5 times.

The color was not noted until the specimen had been put into formalin. Above the mid-lateral region were 7 vertical bands made up of 2 short bars, one above the other, or in some cases of 4 short bars arranged in series of 2 pairs (as if the single pair had merely been doubled). The arrangement of these bars is shown in Plate III, figure 16. Below the mid-lateral region was a row of 9 black spots (on the left side 10), and under these on the belly 3 spots.

This little fish was found clinging to the rocks by its characteristic ventral sucker, which is formed of the two confluent pelvic fins and which is free from the abdomen. Another characteristic feature or structure of this fish was the fringe of filaments on the upper or inner edge of each pectoral fin. In this specimen there were 10 on each fin; some of these were single, having one filament on one base, and some double. It is to this fringe that Evermann & Marsh refer, when they say of the pectoral, "upper rays partially detached and silk-like." These fringing structures are portrayed in figure 17, Plate III.

XXV. Family Echeneididæ. The Sucking Fishes.

The Echeneids are generally slender, much elongated fishes, especially characterized by the presence on the back of the head and shoulder region of a sucking disk which morphologically is a transformed spinous dorsal fin. The morphology of this disk has been studied attentively, the most recent and thorough research being that of Reinhard Houy.[1] The mode of formation of this disk can be gotten only by studying its development. The procuring of such material is obviously a matter of great difficulty. However, a beginning has been made. The pelagic eggs have been collected and identified by Delsman.[2] Larval forms of *Remora remora* as short as 5.6 mm. and of *Echeneis lineata* 14 mm. long, from the towings of Johann Schmidt's expeditions in the north Atlantic are being studied by A. Vedel Tåning.[3] When the microscopical studies of his sections are published, we may expect to learn something of the manner of formation of the sucking disk of the Echeneids.

The habit common to fishes of this family, of attaching themselves to sharks and other large fishes by their dorsal suckers, arises probably from two causes. They are, generally speaking, poor swimmers and as such they do not easily procure food. Attached to sharks, large barracudas and groupers they are sure of both transportation and food (in the form of fragments from the large fish's prey).

A special modification of this general habit I have treated elsewhere.[4]

[1] Reinhard Houy. Beiträge zur Kenntniss der Haftscheibe von *Echeneis*. Zoologische Jahrbücher (Anat. Abth.), 1910, vol. 29, pp. 101-138, 4 pls.

[2] H. C. Delsman. The Eggs of the Suckerfish *(Echeneis naucrates)*. Nature, 1926, vol. 118, p. 805.

[3] A. Vedel Tåning. Position du Disque Céphalique chez les Echénéides au Cours de l'Ontogenese. Comptes Rendus Académie Sciences, Paris, 1926, vol. 182, pp. 1293-1295, 2 figs.

[4] E. W. Gudger. An Odd Place of Refuge. The Habit of the Shark Sucker, *Echeneis* or *Remora*, of Taking Shelter in the Gill-chamber or Mouth-cavity of its Host. Natural History, 1922, vol. 22, pp. 243-249, 1 fig.

67. *Echeneis naucrates* Linnæus. Shark Sucker, Sucking Fish. Jordan & Evermann, III, 1898, p. 2269, pl. cccxxix, fig. 796; Evermann & Marsh, 1900, pp. 301-302, fig. 94; Jordan & Thompson, 1905, p. 254.

This is the only Echeneid identified by me at Tortugas or Key West, although the brown form, *Remora,* was seen at the latter place. However, at Tortugas, on one occasion, I captured by trolling a large barracuda and from it got a small striped sucking fish about 4 inches long and having a plumose tail. This little fish was taken to the laboratory and, as the hour was late, placed in an aquarium of running salt-water to await examination and identification next morning. But when morning came the fish had mysteriously disappeared. The Cuban ichthyologist, Poey, described in 1856 a little 3-inch *Echeneis,* having 10 lamellæ in the disk, striped sides and a plumose tail, as *Echeneis sphyrænarum,* "the sucker of the barracudas." This fish Poey affirmed to be found only adhering to barracudas. Whether or not my small specimen was identical with Poey's can not be stated, since no count was made of the lamellæ of its disk.

Through the kind help of Peter Roberts, keeper of the fish market at Key West, it has been possible to obtain a remarkable collection of small specimens (34 fish ranging from 70 to 123 mm., 2.75 to 4.88 inches, in standard length). The smallest of these were studied in a paper published two years ago.[1]

Utilizing larger specimens and considerable data obtained at Tortugas and elsewhere, it is planned to work up papers on the variation of the lamellæ of the sucking disk, and on the life history of the fish.

XXVI. Family Blenniidæ. The Blennies.

This is a large family, having about 80 genera and over 400 species, found in all marine waters, tropical, temperate and arctic. These fishes are carnivorous, are generally small and of no economic importance. Only one species was collected, but it is a form new to the Tortugas.

68. *Rupiscartes atlanticus* (Cuvier & Valenciennes). Jordan & Evermann, III, 1898, p. 2397; pl. cccxxxix, fig. 825.

One specimen, 3.75 inches over all, had dorsal fin rays XII, 21; anal 23. Jordan & Evermann give XII, 21 or XIII, 20 for the dorsal; and 24 or 25 in the anal; while their figure has 34 rays in the dorsal (17 of these being apparently spinous and 17 soft rays) and 23 in the anal fin.

Being absent when this fish was taken, I can give its colors only as a fresh formalin specimen. The general color was black. The dorsal was black with an orange edge, wider in front than behind, anal also black. The caudal was orange-red above, dark in the central part and light orange below. The pectorals orange-colored, lower half of rays orange-red. Lower lip orange in middle region, *i. e.,* about halfway between point of jaw and corner of mouth.

Pectoral fins about equaling length of head, but not quite reaching to the vent—lacking about one-fifth of total distance from anterior base of pectorals to vent—not "reaching past vent." Mouth with fimbriated lips which retreat like a shark's, revealing the comb-like teeth. Two large posterior recurved canines in the lower jaw fitting into cavities in the roof of the mouth. This fish had a nearly vertical forehead and hence was presumably a male. Between the eyes on each side of the median line was a group of

[1] E. W. Gudger. A Study of the Smallest Shark Suckers (Echeneididæ) on Record, with Special Reference to Metamorphosis. American Museum Novitates, 1926, No. 234, 26 pages, 6 text-figs.

short tentacles—6 in number. A group of 2 tentacles midway between each eye and the anterior base of the dorsal—not shown in Jordan & Evermann's figure, which was probably made from a poorly preserved specimen. Lastly, above each eye, not in the iris as shown in figure 825 (J. & E.), there was a single orange-colored tentacle.

This beautiful little blenny is very abundant on the reefs where it hides in the crevices of the rocks and is very hard to catch. Its generic name, applied by reason of its habits, means one that leaps from rock to rock. It is new to the Tortugas and to the waters of the United States.

XXVII. Family Fierasferidæ. The Pearl Fishes.

These fishes have a long, slender, compressed body, mainly tail, the vent being placed in the throat region. They have the interesting habit of living as commensals with the pearl oyster and certain large echinoderms. The Tortugas form is:

69. *Fierasfer affinis* (Günther). Pearl Fish. Jordan & Evermann, III, 1898, pp. 2495-2496; Jordan & Thompson, 1905, p. 255.

A number of specimens was obtained, all from the bodies of a large holothurian, common on the sandy flats in sheltered parts of the lagoon. These latter were placed in pails of water and brought to the laboratory. As the water became stagnant the Fierasfers would come out. I never succeeded in catching them in the act, but Professor Linton,[1] working at Tortugas some years before, saw them both emerge from and re-enter their place of abode. My rather considerable data on this curious fish will be reserved for another and fuller paper.

XXVIII. Family Antennariidæ. The Frog Fishes.

The fishes of this highly specialized family inhabit tropical seas. They are very bizarre in form and very interesting in habits. Poor swimmers, and hence generally inactive, most of them live in Sargassum weed, to which they hold fast by their curious hand-like pectorals. The presence of dermal filaments and their remarkable adaptive coloration make difficult the detection of these fishes in the floating seaweed.

70. *Pterophryne gibba* (Mitchill). Mouse Fish, Sargassum Fish. Jordan & Evermann, III, 1898, p. 2717; Evermann & Marsh, 1900, p. 334; Jordan & Thompson, 1905, p. 255.

Ten or a dozen specimens were collected in gulfweed (Sargassum) in Southeast Channel after a blow. They varied in length from 1.75 to 3.25 inches. All were kept in aquaria and were studied at intervals for 17 days. Some of these laid eggs imbedded in a gelatinous egg-raft similar to that observed by me as laid by *P. histrio* in the Beaufort, North Carolina, laboratory of the United States Bureau of Fisheries, in 1905.[2]

Having fairly extensive notes on the structure, coloration and habits of this interesting fish, I purpose later writing a somewhat longer account than will fit in here.

[1] Edwin Linton. Note on the Habits of *Fierasfer affinis*. American Naturalist, 1907, vol. 12, pp. 1-4, 2 figs.

[2] E. W. Gudger. A Note on the Eggs and Egg-laying of *Pterophryne histrio*, the Gulfweed Fish. Science, 1905, n. s. 22, pp. 841-843.

CONCLUSION

In the course of my work at Tortugas, 70 Teleosts, belonging to 28 families, have been studied. Apart from the very great diversities in structures and habits which have been recorded herein, two things from the standpoint of the taxonomist stand out prominently. The first is that basing the classification of tropical fishes on coloration is a very dangerous thing. Most of the fishes in Tortugas, as noted frequently above, have from two to five color phases in life and, even when studying the fish in a state of comparative quiet in an aquarium and when it is presumably normal, it is very difficult to determine which is its normal color phase.[1] When the fish dies, its color changes either entirely or at any rate in its intensity, so that the coloration of the dead fish is often markedly different from that of the live one. It is equally dangerous to describe and to classify a tropical fish from a single specimen, since these fishes are so very variable in the number of fin rays, in the relative proportions of the body, in scale count, and in the many details which help to distinguish one species from another. These points have been called to the reader's attention in nearly every species considered in this paper, and are here recapitulated for the sake of emphasis.

[1] In this connection it is interesting to note that Dr. C. H. Townsend, director of the New York Aquarium, has been studying the color phases of tropical fishes in captivity over a number of years and has had made a series of colored paintings of such color changes. Some of these have been published in current issues (1928) of the Bulletin of the New York Zoological Society. These and others (some 28 or 30 in all) will later appear in an extensive article on the subject in Zoologica: Scientific Contributions New York Zoological Society.

PLATES

Plate I

Fig. 1—Diagrams of jaws of *Lycodontis vicinus;* A with the hinder jaws brought close together, B expanded. The shaded regions are the jaws, the unshaded region between represents the folds of skin which act as a breathing valve to prevent regurgitation of the water.

Fig. 2—Head of *Lycodontis funebris* drawn from the side, showing mouth, teeth, eye, and sensory pores.

Fig. 3—Diagrammatic sketch of transverse section of pharynx of *Lycodontis funebris* showing 4 pads of supra- and infra-pharyngeal teeth surrounding the gullet.

Fig. 4—The pear-shaped heart of *Lycodontis funebris* showing the ventral aorta with its four branches on each side, each with its very much reduced gill cleft.

Fig. 5—The alimentary canal of *Lycodontis funebris* seen in dorsal aspect. Note the slight constriction between œsophagus and stomach, the long pouch-like stomach dwindling into an appendix posteriorly, and the intestine coming off the stomach just behind the œsophageal constriction and displaced to the right.

Fig. 6—Heart-shaped spot on back of head of *Jenkinsia stolifera.*

Fig. 7—Showing swarm of *Jenkinsia stolifera* around and above the gray snapper, *Neomœnis griseus,* but leaving a clear space immediately over the snapper.

Fig. 8—Eye of *Tylosurus marinus* showing curious shape of iris.

Fig. 9—Right lobe of liver of *Tylosurus raphidoma* showing the imbedded gall bladder.

Fig. 10—Angle of opercle of *Holocentrus siccifer* showing twin spines.

Fig. 11—Portion of spinous dorsal of *Holocentrus siccifer,* showing the yellow spines, the transparent and the red portions (here shown in black) of the membrane joining these.

Fig. 12—Diagrams of mouth of *Holocentrus tortugæ,* showing (A) the outline when closed, and (B) the same when widely opened. This enormous increase of the gape enables the fish to swallow relatively large objects.

Fig. 13—Shows the arrangement of clear and red portions of the spinous dorsal of *Holocentrus tortugæ* beginning at spine IV.

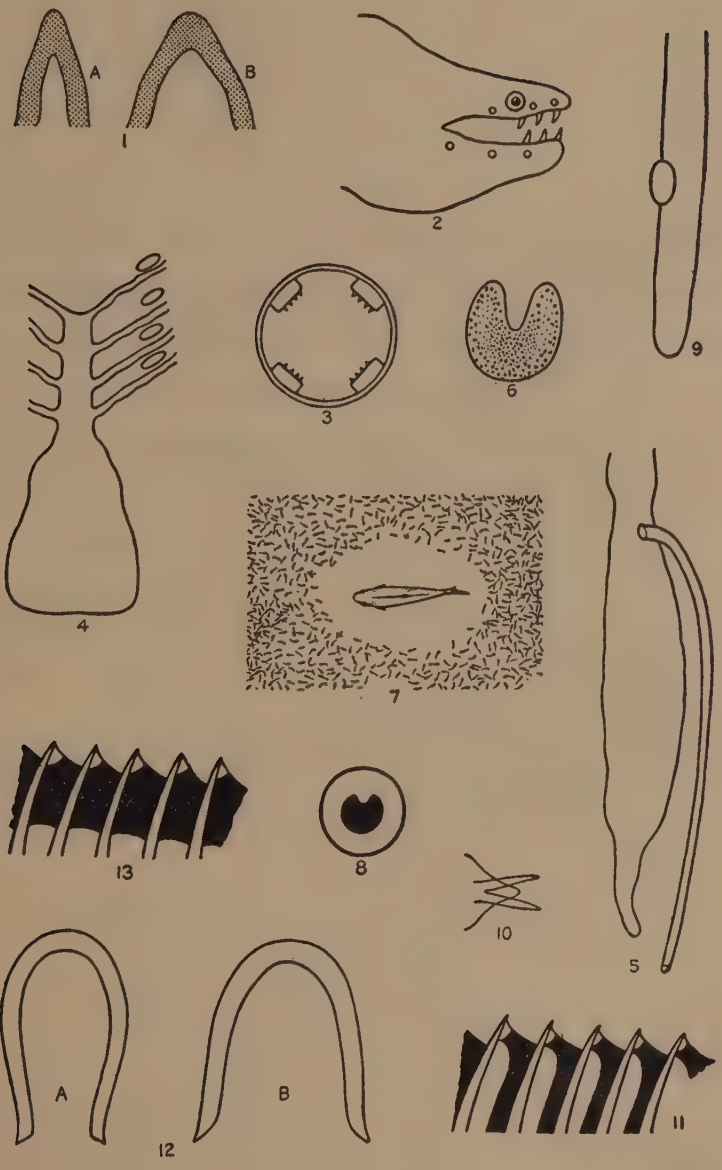
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Plate II

Fig. 1—The alimentary canal of *Gymnosarda alleterata* seen in ventral aspect, and somewhat uncoiled.

Fig. 2—Caudal peduncle and caudal fin of *Caranx bartholomæi* showing the stumpy appearance and how the serrated lateral scutes appear from above.

Fig. 3—The deeply forked caudal fin of *Caranx bartholomæi*—made from a tracing of the actual fin.

Fig. 4—Shows the malformed upper lobe of the caudal fin of *Trachinotus glaucus*. This has probably been bitten off.

Fig. 5—Eye of *Apogon sellicauda* showing horizontal dark band across iris and pupil.

Fig. 6—Caudal fin of *Apogonichthys puncticulatus* showing the rounded notch in the central posterior edge.

Fig. 7—Posterior part of the body of *Epinephelus morio* showing the enormously distended abdomen due to the presence of an 11.5-inch snapper in an 8-inch stomach.

Fig. 8—Head of *Hypoplectrus unicolor puella* showing the blue bands on head and around eye. To one side is shown the eye with its completely encircling blue band.

Fig. 9—Sketches of the nasal openings of *Priacanthus cruentatus* showing the relative size of the anterior and posterior nares.

Fig. 10—The stomach of *Neomænis analis* showing œsophagus above and beginning of small intestine on left. Contrast this stomach with that in *Gymnosarda alleterata* in figure 1, Plate II.

Fig. 11—Body and tail of *Hæmulon plumieri* to show sharp downward bend of lateral line on a line joining hinder edge of soft dorsal and last spine of anal.

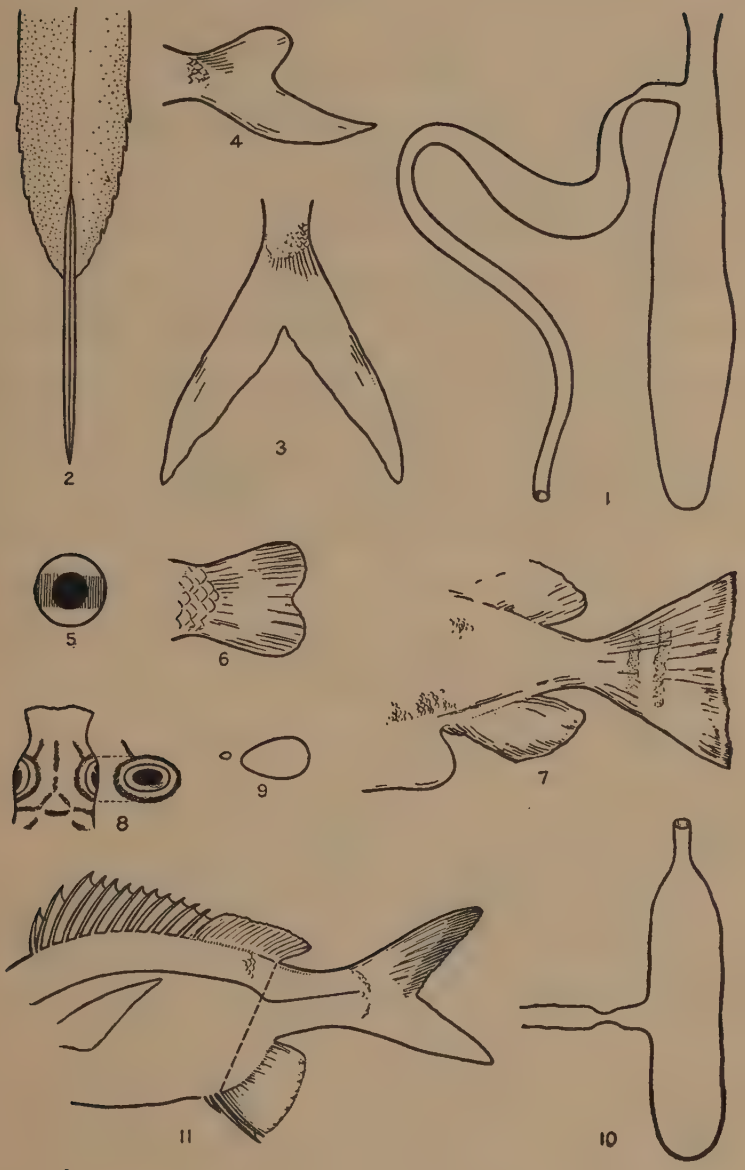
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Plate III

Fig. 1—Anterior part of the alimentary canal of *Kyphosus sectatrix* showing the crop-like pouch and the enlarged stomach-like cavity in the intestine.

Fig. 2—Longitudinal vertical section through body of *Kyphosus sectatrix* to show the outline of the abdominal cavity. This is very high anteriorly, reaching nearly to the backbone, but dropping down very abruptly to the anus behind.

Fig. 3—*Iridio bivittatus* diagrammatically shown in the flat S-shaped curve which it assumes in order to keep from falling over when resting on the sand.

Fig. 4—Pelvic region of *Sparisoma abildgaardi* showing the 3 large overlapping scales between the pelvic fins.

Fig. 5—A lateral scale from about the middle region of the body of *Sparisoma abildgaardi*. Note the deliquescent structure of the "tree." The "tree" in the posterior scales is excurrent.

Fig. 6—A lateral line scale from the hinder region of *Sparisoma viride*. Note the excurrent form of the "tree"—a main trunk with branches.

Fig. 7—Lateral line plexus in region of 18th scale (counting backward) on right and left sides of *Sparisoma cæruleus*. Similar plexuses were found on the red parrot fish.

Fig. 8—Median tooth of *Pomacanthus arcuatus*. (Natural size, 5 mm. high by 0.5 mm. wide.)

Fig. 9—Posterior region of *Pomacanthus arcuatus* showing dorsal and anal fin filaments, the latter being doubled in this specimen.

Fig. 10—A multi-cusped tooth of *Teuthis hepatus*, adapted for nibbling and browsing on algæ and corallines.

Fig. 11—Eye of *Teuthis hepatus* showing the blue and orange bands crossing the iris.

Fig. 12—Semi-diagrammatic figure of lancet, muscles, and spinal column in *Teuthis bahianus*.

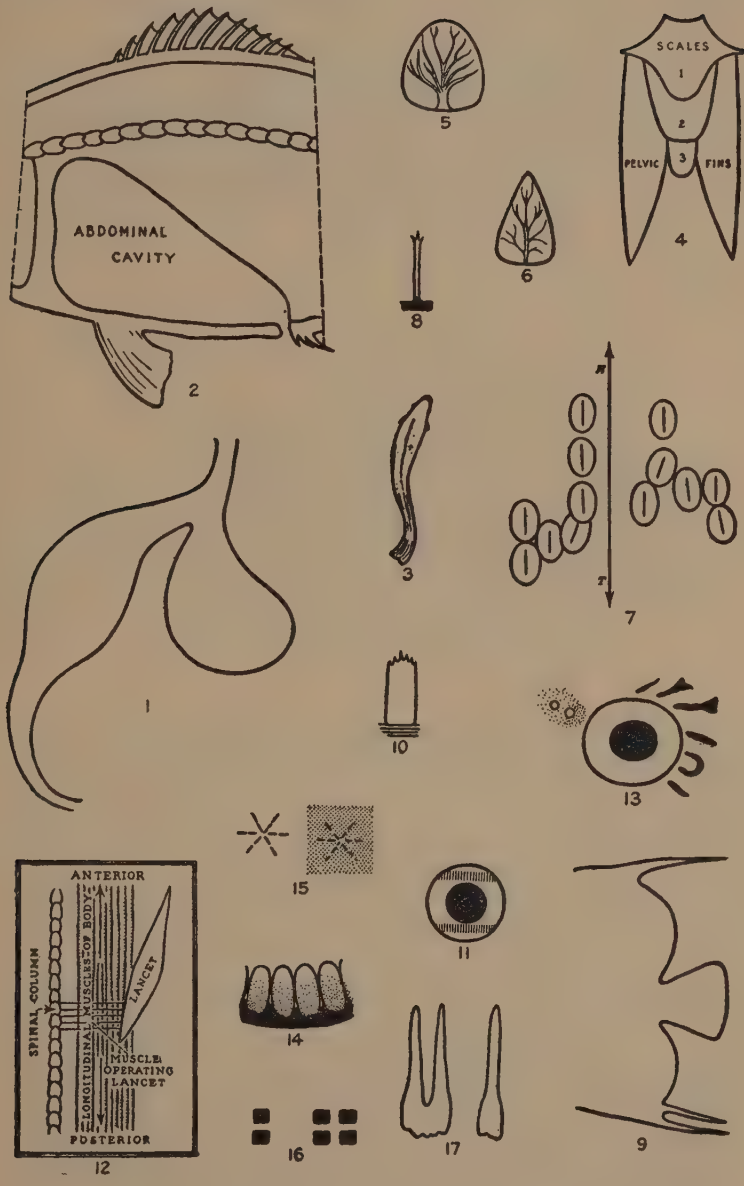
Fig. 13.—Eye with markings, and double nostrils of *Teuthis bahianus*.

Fig. 14—Teeth of *Lactophrys triqueter* showing how they are set in the gum.

Fig. 15—This figure gives the detail of the stellate spots on the carapace of a young *Lactophrys bicaudalis*. For a photograph of the fish see Plate IV, figure 2.

Fig. 16—Short square bars on sides of *Gobius soporator* arranged either in pairs or in fours.

Fig. 17—Filaments from fringe on upper edge of pectoral fin of *Gobius soporator*.


ABDOMINAL CAVITY
SCALES
1
2
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PELVIC FINS
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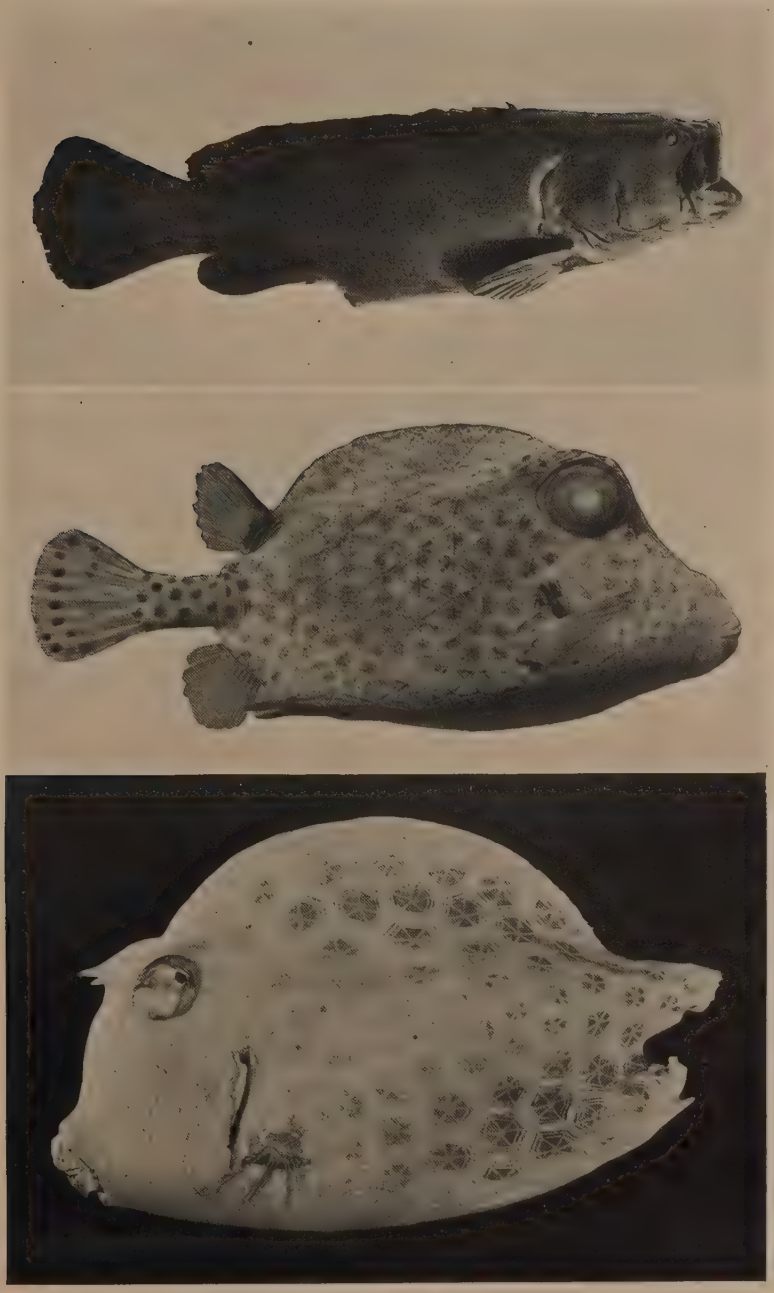
ANTERIOR
SPINAL COLUMN
LONGITUDINAL MUSCLES OF BODY
LANCET
MUSCLE OPERATING LANCET
POSTERIOR

Plate IV

Fig. 1—*Promicrops guttatus*. Jew fish. 7 feet long and over 320 pounds in weight. Photographed by Alfred G. Mayor.

Fig. 2—Photograph of young *Lactophrys bicaudalis*, made from alcoholic specimen. 3.6 inches (nat. size).

Fig. 3—Trunk of trunk fish, *Lactophrys tricornis*, taken from stomach of a shark at Tortugas.



VI

OBSERVATIONS ON CERTAIN LITTORAL AND TERRESTRIAL ANIMALS AT TORTUGAS, FLORIDA, WITH SPECIAL REFERENCE TO MIGRATIONS FROM MARINE TO TERRESTRIAL HABITATS

By A. S. Pearse

Three text-figures

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OBSERVATIONS ON CERTAIN LITTORAL AND TERRESTRIAL ANIMALS AT TORTUGAS, FLORIDA, WITH SPECIAL REFERENCE TO MIGRATIONS FROM MARINE TO TERRESTRIAL HABITATS

Palæontological evidence indicates that the ocean was the first home of animals, and that through the ages migrants from it have gradually populated land and fresh-water habitats. In the ocean there is more or less thermal stability, and there animals live in a medium which is much like the fluids within their bodies. The oxygen content is rather low compared to that in the atmosphere, but is on the whole more constant than in fresh water. The carbon dioxide is usually much higher in littoral marine habitats than in the atmosphere (McClendon, 1918).

Animals which have migrated from the ocean into fresh water must endure greater variations in temperature, oxygen, pH and mineral salts, and may live in habitats where the amount of available water fluctuates. Those which have attained to life on land live in a rarer medium, which permits more rapid movement, and can carry on metabolism at a higher level because there is an abundance of oxygen available. They must, however, endure extreme variations in temperature and may be in danger of death from extreme desiccation. The respiration of any animal takes place through moist membranes, and residents in aquatic habitats acquire oxygen through the external surface of the body or through gills. Those animals which have taken up terrestrial life show a tendency to leave off gill- and skin-breathing. They respire more and more through the walls of internal cavities, and thus conserve water as well as gain protection for their respiratory membranes.

In the past, various types of animals have migrated from the ocean into fresh-water streams or lakes, and from there gained a foothold on land. Others have reached the same goal more directly through the tidal zone. Such migrations are going on today. On the shores of all oceans, animals may be found which are partially adjusted to life in fresh water or on land.

The Dry Tortugas is an isolated group of seven small islands, or keys, situated on the border of the Gulf Stream about 63 nautical miles west of Key West, Florida, and 42 miles from the Marquesas, another group of keys which is nearer the Florida coast. They are composed of sand and coral rock and are surrounded by coral reefs. They contain no fresh water, except that in the small, closed reservoirs constructed by man, and therefore offer an excellent opportunity to study littoral animals which have no immediate contact with fresh-water habitats. In fact, the sea-water at Tortugas is somewhat more saline (36.01) than the open ocean (35.02), according to Dole (1914).

From July 11 to August 19, 1928, the writer studied the animals on the reefs, rock beaches and land at Tortugas. Most of the observations were made on animals collected on or near Loggerhead Key, the largest of the islands, and on Garden Key and the reefs near it. Observations were concerned largely with crabs and hermit-crabs, but a few were made on other reef animals.

ABILITY OF CERTAIN LITTORAL AND LAND ANIMALS TO LIVE IN FRESH WATER

In the laboratory various animals were kept in open glass containers of various sizes. These stood on tables at ordinary air temperatures, which ranged from 27.3° to 34.6° C. The water which came from the laboratory storage tanks had a temperature of 31.5° C., or more. Water standing in open dishes in the laboratory tables had a temperature a degree or more below that of the air, depending on wind, moisture saturation deficit in the atmosphere, temperature and other factors. For example, on July 16 temperatures in the laboratory were as follows: air, 31.05° C.; mixture in finger bowl of 0.25 sea-water and 0.75 rain-water, 28.90° C.; mixture in finger bowl of 0.75 sea-water and 0.25 rain-water, 28.90° C.; sea-water in finger bowl, 29.04° C. On July 24 at 2 p.m. the air was 33.8° C. and sea-water in a finger bowl was 31.0° C. The rain and sea-water used for all experiments came from closed, wooden storage tanks, and was changed in the dishes at least once during every 24 hours.

The results of the experiment are shown in Table 1. Among the animals which appear to be least able to live in fresh water are (1) those from the coral reefs—the stomatopod (*Gonodactylus*) and the oxyrhynchid crabs (*Mithrax* and *Microphis*); and (2) also the more or less pelagic portunid crabs (*Portunus* and *Callinectes*). The animals that show a little greater degree of toleration for fresh water are a little snail (*Cerithium*), the smallest grapsoid crab (*Pachygrapsus*), the smallest hermit-crab (*Clibanarius*), the largest coral reef snail (*Strombus*), and the ghost crab (*Ocypode*). Those which show the greatest degree of toleration are the larger hermits (*Calcinus, Cenobita*), a grapsoid crab (*Geograpsus*), the land crab (*Gecarcinus*), the large reef snail (*Astræa*), and the two small snails (*Nerita, Litorina*) which frequent the high-tide mark on rock beaches.

The animals on the coral reefs and from the open sea have little toleration for fresh water, but those on rock beaches show more. Large size (*Strombus*) may enable one of the former to live for some time in fresh water. The snails (*Litorina, Nerita*) from the rock beaches are probably more resistant to fresh water than those from the coral reefs, because they can remain quiescent and exclude all external media for considerable periods of time. The land hermit (*Cenobita*) and crab (*Gecarcinus*) have apparently, in acquiring the ability to live on land where they must conserve water, also become able to some extent to resist the loss of salts from their

body fluids into the surrounding medium. The ghost crab (*Ocypode*), though about the same size as the land crab (*Gecarcinus*), does not live in fresh water nearly as long. This difference is perhaps concerned with the greater dependence of the former on the lining of its branchial cavity for respiration and its greater activity.

Some species of portunid crabs, which have contact with fresh or brackish water, are apparently able to live indefinitely in the former. The blue crab (*Callinectes sapidus* Rathbun) is frequently found in perfectly fresh water, and at Beaufort, North Carolina, the writer has found it in streams with crayfishes and typically fresh-water fishes like the pumpkin-seed, *Eupomotis gibbosus* (Linnæus). Another portunid, *Callinectes ornatus* Ordway, has been kept for three months in fresh water (Brues, 1927). Such crabs are in sharp contrast to those studied at Tortugas.

The first time *Gecarcinus lateralis* was tested the solutions were low enough in the glass dishes so that the crabs, by standing on the legs on one side and stretching upward, could get all or part of the body into the air. It will be noticed (Table 2) that these crabs lived much longer than a second lot in which it was not possible for the crabs at any time to get their bodies out of the water. Small specimens of *Ocypode albicans* lived longer in solutions, but for a shorter time in air than large individuals. Apparently the size of a crab has a more or less direct relation to the length of time it can conserve moisture and live in air, but young crabs have greater ability to keep pace with marked changes in the salinity of the surrounding medium.

The salts in the blood of marine crustaceans vary with differences in the salinity of the surrounding medium, but are always a little less in amount. The same holds true for many other invertebrates studied, except starfishes and sipunculids which have blood of about the same salt content as the surrounding ocean. The osmotic pressure of the blood of marine invertebrates and cartilaginous fishes is the same as that of the surrounding medium, except for the fact that some sharks are hypertonic (Duval, 1924). In brackish water there are animals showing three types of adjustments to the salinity of the surrounding medium (Redeke, 1922): (1) oligohalinophil, (2) mesophalinophil and (3) polyhalinophil. These are also classified as stenohalines and euryhalines (Duval, 1925). Three conditions may occur in relation to equilibria between the body fluids of marine animals and the surrounding medium (Fredericq, 1922): (1) osmotic and salt, (2) osmotic only and (3) independence. The effects of varying salinity may thus result in isotony, in changes in particular salts, or produce no effect in body fluids. Some animals can not endure changes in the salinity of the surrounding medium, and others are euryhaline but very susceptible to changes in the balance in the salts in their medium (Adolph, 1925).

Many typically marine animals, such as *Balanus, Patella, Purpura, Cardium, Ostrea* and *Mytilus,* will live in fresh water (Flattely and Walton,

1922). These are often estuarine types, but more than this ability is necessary to enable them to become permanently established in fresh water (Annandale, 1922). Some such animals require marine conditions for breeding and some are limited by other causes.

Of course the writer does not believe that the results of his experiments actually represent the extreme limits of all the animals tested to endure the conditions to which they were subjected, but they probably do give a correct idea of the relative abilities of the types studied. In general the animals which show the greatest degree of toleration for fresh water are also those which withstand desiccation longest. The largest animals usually survive longest in air and in fresh water. Those which have special abilities for enduring desiccation are also often able to survive prolonged submergence in fresh water. This does not mean that migration into fresh water or on to land are related, but that the acquisition of external membranes which resist changes and thus maintain stability in the internal fluids makes an animal resistant in any medium which differs from that to which it has been acclimated. The beach hopper (*Orchestia*) lives for long periods in air but can not long endure fresh water (King, 1914). A ghost crab, *Ocypode ceratophthalmus* (Pallas), on the Pacific Islands lives between tides and dies after 2 hours in fresh water (Gardiner, 1903); at Tortugas, *O. albicans* Bosc. lives mostly above high-tide mark and can survive for as much as 18 hours. *Orchestia* has apparently developed ability to endure lack of water, but not variation in salinity. *Ocypode* has some degree of resistance to both types of changes. In Table 1 it is apparent that practically all the animals tested live longer in air than in fresh water. This probably means that it takes longer to lose enough water than to lose enough essential salt constituents to cause death. If any of the animals lived longer in fresh water than in air, the fact could be interpreted as indicating that such animals in their evolution probably first became adjusted to fresh water and then to land, but at Tortugas there are no such examples. Colgan (1910) also found that the snails on rock beaches lived considerably longer in air than in fresh water. Probably such animals migrated, and are migrating, directly from the ocean through the intertidal zone to land.

ABILITY OF CERTAIN LITTORAL AND LAND ANIMALS TO ENDURE DESICCATION

There are many land animals which can live wholly without water, except that which they obtain from their food (Babcock, 1912; Buxton, 1923), or can endure long periods without contact with water. At present there are many animals becoming adjusted to life on land, and they show varying degrees of ability to live for longer or shorter periods in air. Along the rock beaches on the coast of Ireland the snails of the genus *Litorina* are arranged in zones.

High on the beach live the viviparous *L. neritoides* Lamarck and *L. rudis* (Donovan); intermediate is *L. obtusata* Linnæus which produces eggs that soon hatch out second-stage veligers; and near low-tide mark lives *L. litorea* Linnæus which lays encapsuled eggs that hatch out early veligers. The beach crabs show an interesting series: mycterids live at low-tide mark, fiddlers are intertidal and close their burrows when the sea covers them, but always carry sea-water in their gill cavities; ghost crabs live on land and carry no water, though they often visit the sea; many land crabs live far from the ocean, and return to it only once each year to breed. In Siam there are several species of fishes which live for 3 to 4 months without water, buried in the earth in dry fields, from which they are commonly dug out with spades by the natives (Smith, 1927).

In becoming adjusted to land, an animal must acquire the ability to respire air through moist membranes, which are usually enclosed within internal cavities, instead of through water-bathed gills or cutaneous membranes. It must also build up some ability to resist loss of water, and this is usually aided by some sort of impervious body covering. At Tortugas the hermit crabs and crabs furnish an interesting series of transitions from

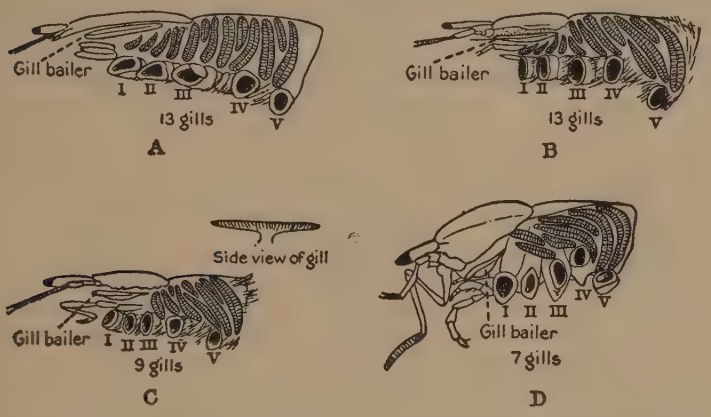

FIG. 1—Gills of hermit crabs. (*A*) *Paguristes puncticeps* Benedict; (*B*) *Calcinus sulcatus* (H. M.-Edwards); (*C*) *Clibanarius tricolor* (Gibbes); (*D*) *Cenobita diogenes* (Latreille).

marine to terrestrial life, and these are correlated with ability to live in air. Four species of hermits were studied:

Paguristes puncticeps Benedict is a large species which lives at depths of 0 to 2 or more fathoms. It has 26 gills.

Calcinus sulcatus (H. M.-Edwards) is a medium-sized species which is usually found just below low-tide mark, but invades the intertidal zone when conditions are favorable. It also has 26 gills.

Clibanarius tricolor (Gibbes) is a little hermit which frequents rocky shores and creeps about or clings in crevices near high-tide mark while waves dash over it. It has 18 gills.

Cenobita diogenes (Latreille) is of considerable size. It lives on land and during the day hides under various objects or often even wriggles down into loose soil. It has 14 gills.

The segregation of *Calcinus sulcatus* and *Clibanarius tricolor* into separate habitats is nicely shown by their local distribution on Loggerhead Key. The prevailing wind strikes the east side of the island and casual observation shows that *C. sulcatus* is rare and that *C. clibanarius* is abundant there. On the west side the quieter water is often occupied by *C. sulcatus*. On August 9, 1928, there was a southerly wind all day which blew straight from one end of the island to the other and thus made the waves on the east and west sides of about equal strength. At 10^h 30^m a.m., at low tide, hermits were collected from as turbulent water as possible on each side with the following results: from the east, 146 *Clibanarius tricolor;* from the west, 128 *Clibanarius tricolor*, 8 *Calcinus sulcatus.*

On August 15 low tide was at 4^h 30^m p.m. and the wind was easterly. One hundred hermits were collected on the west from low-tide mark to a depth of 2 feet at exactly the same locality as on August 9; all were *Calcinus sulcatus.* At low tide, 5^h 32^m p.m. on August 17, the writer collected on the east side of the island at the same locality as on August 9. A gentle east wind was blowing. The largest hermits were collected from the deepest and quietest water. The results were 113 *Clibanarius tricolor* and 1 *Calcinus sulcatus.*

As the hermits progress toward land life the number of gills is reduced and the soft, vascular abdomen takes more part in respiratory activities. Correlated with these changes are modifications in the circulatory system. Gardiner (1903) states that *Cenobita* will live without its gills. The writer removed the gills from three cenobitas on August 6. One escaped from its dish on the next day, but the other two lived until August 15, when they were preserved in alcohol and later examined to make sure that none of the gills remained. One of the latter was found to have remnants amounting to about two and a half gills on one side of its body, but there was none in either of the branchial cavities of the other hermit. Both these hermits remained more or less active after they had been operated upon. They walked about at night, wriggled down into the loose sand in their dish, and ate food.

As the preliminary tests concerning how long hermit crabs could live in clean dry glass finger bowls without water were not conclusive, a long series of tests was made. Considerable numbers of hermits were brought into the laboratory and put in sea-water after various intervals of time. In most cases larger individuals survived longer than smaller.

Clibanarius tricolor, after 12 to 18 hours in air, showed 21 to 86 per cent of recoveries when about 50 individuals were placed in water each hour; 19 to 24 hours, 8 to 29 per cent; 25 to 30 hours, 0 to 7 per cent; 31 to 36 hours, 0 to 12 per cent; 37 to 42 hours, 2 to 8 per cent; 43 to 48 hours, 0 to 7 per cent; 49 to 54 hours, 0 to 3.3 per cent; 55 to 60 hours, 0 per cent.

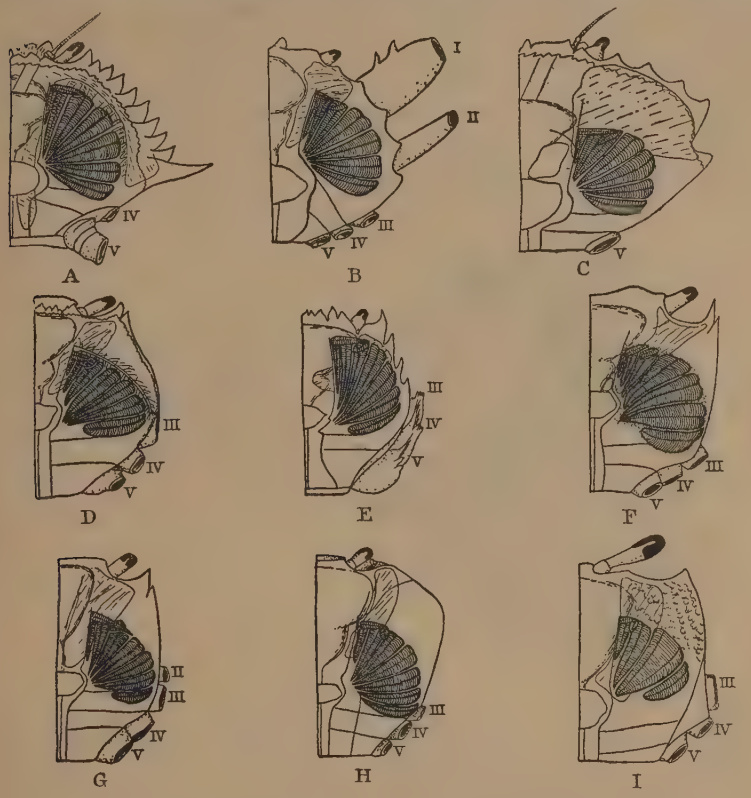

FIG. 2—Gills of crabs. (*A*) *Callinectes marginatus* A. Milne-Edwards; (*B*) *Mithrax sculptus* (Lamarck); (*C*) *Leptodius floridanus* (Gibbes); (*D*) *Grapsus grapsus* Linnæus; (*E*) *Plagusia depressa* (Fabricius); (F) *Pachygrapsus transversus* Gibbes; (*G*) *Geograpsus lividus* Milne-Edwards; (*H*) *Gecarcinus lateralis* (Freminville); (*I*) *Ocypode albicans* Bosc.

Less extensive tests were made with *Calcinus sulcatus* and, as this species was harder to obtain, only 5 or 6 individuals were placed in water each hour. Small individuals did not survive as long as those of *Clibanarius tricolor* but large individuals survived longer. With the latter typical results were as follows: 50 hours, 83 per cent; 64 hours, 60 per cent; 68 hours, 0 per cent; 69 hours, 40 per cent; 70, 71 and 72 hours, 0 per cent. A large

Calcinus with its snail shell had a volume of about 10 c.c., while a large *Clibanarius* had a volume of about 1 c.c.

The crabs at Tortugas, like the hermits, also show modifications in structure which are correlated with ability to live on land. There is in this case no progressive reduction in the number of gills, but the volume of the gills in relation to the volume of the body was found to decrease steadily (Table 2). To determine this, crabs were hardened in 1 per cent chromic acid for 24 hours; the legs were then removed, the carapace separated from the body, and the gills carefully cut away from their attachments. All parts of the body were allowed to remain on blotting paper for five minutes. The gills were then dropped into water in one graduate glass and the remaining parts of the body into that in another.

FIG. 3—Ventral view of branchial cavity of *Ocypode albicans* Bosc., showing distribution of branchial tufts; x 1.5.

The gills of *Callinectes marginatus* are reduced in number (16). All other crabs studied, except *Ocypode*, had 18 gills. *Gecarcinus lateralis*, though well adjusted to life on land and probably not visiting the ocean except to breed, shows no reduction in the number of its gills (18), but the membranous walls of its branchial chamber are quite vascular. *Ocypode albicans* has a respiratory system which is modified considerably for life in the air. The gills are reduced in number (12) and on each side two of them are fused into a great gill which is provided with two pairs of longitudinal vessels (fig. 2). There are also five irregular rows of respiratory tufts (fig. 3) along the blood-vessels on the membranous lining of the branchial cavity. These remind one of those described in *Birgus* by Semper (1878).

ABILITY OF CERTAIN LITTORAL ANIMALS TO ENDURE HIGH TEMPERATURES

Mayer (1914) studied the temperatures at which many reef animals at Tortugas live and determined the limits of toleration for a number of species.

"Tropical marine animals commonly live within 5° C. of their temperature of maximum activity and with 10° to 15° C. of their upper death temperature. . . . Thus the factor of safety in respect to elevation of temperature is far less in tropical than in temperate marine animals, and they are, relatively speaking, poorly adjusted in a physiological sense to their temperature environment."

A temperature of 38° C. on the reef was found to be fatal to many animals.

The writer made no direct observations in the laboratory on the ability of animals to endure high temperatures, but his rather limited field observations are in agreement with Mayer in regard to conditions on the reefs. For example, in bright sun on July 28, 1928, on Bird Key Reef in shallow water over coral rock, the water had a temperature of 38.1° C. There were no living corals, but the following animals were found alive under rocks and in crevices: an anemone; *Ophiocoma echinata* L. Agassiz; a polychæte; *Strombus gigas* L.; *Echinometra viridis* A. Agassiz; and *Calcinus sulcatus* (H. M.-Edwards). In an adjacent shallow pool where the temperature was 37.4° C. there were over twenty species alive, including sponges, snails, a chiton, ascidians, a stomatopod, fishes, podurans, *Mithrax coryphe* (Herbst) and *Calcinus sulcatus* (H. M.-E.).

On July 26 temperatures were taken in various situations on the rocky beach along the east shore of Loggerhead Key from 10 a.m. to 2 p.m., the tide being low at $12^h\ 30^m$ p.m. A light wind blew; the day was sunny, with scattered clouds. The results were as follows:

$10^h\ 05^m$ a.m. Water in pool connected with ocean, 30.8° C.

$11^h\ 00^m$ a.m. Water in pool by a *Callinectes marginatus* A. Milne-Edwards, 31.1° C.

$11^h\ 10^m$ a.m. Beside a *Nerita versicolor* Lamarck resting in a shady dry rock crevice, 35.2° C.

$11^h\ 50^m$ a.m. On top of flat stone under which a *Callinectes* was hiding in sand, 33.8° C.; under stone, 31.8° C. $1^h\ 35^m$ p.m. in sand with the crab, 30° C.

$11^h\ 55^m$ a.m. Beside resting *Litorina ziczac* Gmelen on bare rock.

$12^h\ 05^m$ a.m. Shallow 15-inch rock pool containing active fishes, *Gonodactylus*, *Clibanarius*, etc., 34.0° C.; at $1^h\ 15^m$ p.m., 33° C.

$12^h\ 25^m$ p.m. In 18-inch hole in rock containing *Pachygrapsus* and *Clibanarius* crawling in and out of water; in water 31.7° C.; various parts of damp rock, 34.3°, 33.4°, 33.1° C.; on bare, dry rock, 36.8° C.

$12^h\ 45^m$ p.m. In 6 by 12-inch hole with active *Pachygrapsus* and *Clibanarius*, 37.5° C.

$12^h\ 55^m$ p.m. By *Nerita versicolor*, in moist, shady hole in rock, 28.5° C.

1h 00m p.m. By *Clibanarius* in sun on rock at edge of ocean, 33.2° C.
1h 25m p.m. By resting *Nerita* in dry, open rock crevices, 34.4° C., 34.1° C., 34.4° C.
1h 50m p.m. By *Nerita* in rock crevices, 36.8° C., 36.5° C.
2h 00m p.m. By resting mussel (*Pteria vitrea* Roe) in rock crevice, 36.5° C.

From these examples it is apparent that temperatures on ordinary warm, sunny days are not as high as 38° C. on bare rocky ledges exposed to the full sun along the shore. In all cases the bulb of the thermometer was shaded while temperatures were being taken.

ADJUSTMENTS OF LITTORAL ANIMALS TO LIFE ON LAND

In the mountains of North Carolina, Noble (1927) has observed that several species of salamanders live in the same localities and even in the same streams, but avoid competition by occupying quite specific and different habitats. In all parts of the earth and in all habitats such specialization doubtless occurs more or less and is nowhere more apparent than along the seashore, where most beach animals occur in horizontal zones (Pearse, 1914; Flattely and Walton, 1922; Huntsman, 1918). Such habititic zonation is quite striking among the littoral hermit crabs at Tortugas. The largest aquatic species (*Paguristes punctipes*) is found in deepest water, probably because there is food there and its large size makes it more or less immune to attacks by predaceous animals. The medium-sized hermit (*Calcinus sulcatus*) is found at low-tide mark, and probably on account of its presence there the smallest species (*Clibanarius tricolor*) is found near high-tide mark. The land hermit (*Cenobita diogenes*) escapes all competition with other hermits by wandering far from the shore.

The true crabs also show more or less zonation. In the open ocean are swimming portunids, like *Portunus sulcatus* which never comes to shore; but one of these at Tortugas, *Callinectes marginatus*, often invades littoral habitats and is found under rocks at low tide with proper littoral species. In crevices among corals *Mithrax* is dominant, and a few *Leptodius* and *Pachygrapsus* are found. On rocky shores *Mithrax* is rare but *Leptodius* and *Pachygrapsus* are common in crevices and under loose stones; the former more where the bottom is partly soft and the latter more commonly where the substratum is harder. *Grapsus* and *Plagusia* occur where weathered, wave-washed, rocky shores partake more or less of the nature of walls. *Geograpsus* occurs in sheltered coves where there are many loose stones and drift under which they may hide. *Ocypode* is associated with sand and makes its burrow from high-tide mark as far inland as the little island areas at Tortugas permit. *Gecarcinus* probably does not visit the ocean except to breed. Its burrows have much the same distribution as those of *Ocypode*. However, they are seldom in loose sand in open places, but are found among the roots of grasses and other plants.

Thus hermits and crabs begin their migration to land by segregation into habitats. They do not start to go to the land where they may find new advantages and encounter new dangers; they begin by avoiding competition with nearly related species. After that, every species of animal can not come to possess a terrestrial habitat for its own. A *conquistador* must be able to acquire the ability to breathe air and at the same time conserve water for body fluids. In the tropics all temperatures are high, so there is little difference between littoral waters and the atmosphere. Animals with hard exoskeletons, like snails and arthropods, or impermeable skins, like vertebrates, have been the only really successful invaders of the land. They had water-conserving equipment before they started.

Among the crabs studied at Tortugas, *Ocypode* is the most successful land animal. It is omnivorous, agile, diurnal as well as nocturnal, breathes air through branchial tufts and has lost some of its gills. Probably it does not survive as long in a clean, dry glass container (Table 1) as its slow, rather strictly nocturnal rival, *Gecarcinus*, because of its great activity. It lives rapidly, struggles ceaselessly, and quickly wears itself out. *Gecarcinus* and *Cenobita* come forth at night to wander about in search of food. Both are largely vegetarian and scavengers. Judged by fæcal examinations, neither enjoys such a varied menu as *Ocypode*. Both carry parasitic mites in their branchial chambers at times. Probably such parasites are absent from *Ocypode* because it bathes at intervals in the sea. *Grapsus* and *Plagusia* are flattened, alga-eating rock-dwellers. They cling quietly to rocks and feed while waves dash over them. They live in or out of the water and have the speed which goes with terrestrial life, but belong to rocks and beaches. *Pachygrapsus* and *Geograpsus* have thicker bodies and more space in their branchial chambers for air, but their small sizes limit them to short sojourns on land. They spend much of their time lurking in crevices and are most active when there is little sun. *Leptodius* has many relatives which live on mud flats. It is never found far from muddy or sandy bottoms and is inactive in the intertidal zone when it is not covered with water. *Callinectes* is not really a proper intertidal crab, but being active, swift-swimming and acquisitive, it may rest for a time in many shore habitats. *Cenobita* doubtless took advantage of the fact that it possessed a snail shell covering which served for protection and moisture conservation. As it gradually invaded the land, its gills dwindled and its soft abdomen became more vascular. It is now a proper land animal, but, like *Gecarcinus*, pays homage once a year to its ancestral habitat in order to leave its young for a time in the sea.

To recapitulate, most of the hermits and crabs discussed in this paper are predominantly vegetarian in their food habits, but some are also to a certain degree scavengers. Among the three species which have gone farthest in the attainment of life on land, *Cenobita* and *Gecarcinus* are largely vegetarian, but *Ocypode* is an omnivorous scavenger and beach-comber—swift, alert, keen-eyed, more or less diurnal and aggressive.

Certain crustaceans which have become terrestrial have developed homing instincts. *Ocypode* when molested will return directly to its burrow from considerable distances (Cowles, 1908), and the same is true of fiddler crabs (Pearse, 1914). In observing the return of the land hermits (*Cenobita*) on Garden Key to shelter in the morning, the writer was struck by the air of purposeful haste which many of them showed and thought perhaps they were returning to a particular spot to rest during the day. Three well-patronized hiding places about 50 yards apart were therefore selected on the afternoon of July 20 and hermits in each situation were marked on their snail shells with red, blue or yellow ink. Next morning and twice thereafter the hermits in each locality were examined carefully to see if marked individuals returned to the same spot day after day. The results were as follows:

July 22—Locality where 66 hermits were marked red:
65 unmarked; 9 red.
Locality where 79 hermits were marked yellow:
62 unmarked; 7 blue, 1 yellow.
Locality where 22 hermits were marked yellow:
48 unmarked; 5 yellow.
July 27—Locality where 66 hermits were marked red:
157 unmarked; 2 red.
Locality where 79 hermits were marked blue:
384 unmarked; 3 blue; 3 red.
Locality where 22 hermits were marked yellow:
14 unmarked.
July 29—Locality where 66 hermits were marked red:
41 unmarked; 1 red.
Locality where 79 hermits were marked blue:
407 unmarked; 7 blue; 1 yellow.
Locality where 22 hermits were marked yellow:
6 unmarked.

To travel from the locality where hermits were marked red to that where they were marked blue a hermit had to cross a wooden bridge across a moat and traverse an entrance gallery through the walls of Fort Jefferson. Crabs often made this journey during the night. There is apparently no evidence of homing instinct. A *Cenobita* when day comes "beds down" in the nearest convenient shelter and returns to the same spot more than once only by chance. Many land animals, such as perching birds, select a particular spot and return to this over and over again to rest. The spot may not be markedly better than many others, but, once its adequacy as a resting place is established and a habit of returning to it formed, much time is saved because a new resting place does not have to be searched for daily. The writer knows of no evidence that any crustaceans show homing instincts, except those which have more or less permanent homes, such as burrows.

DISCUSSION AND CONCLUSIONS

An animal which migrates from the ocean and becomes established as a resident on land must not only acquire the ability to breathe air and at the same time conserve water, but also make profound modifications in its locomotor and nervous system (Pike, 1924). Air is a rarer medium than water and limbs must be stronger to bear the body above the ground. Jehu (1923) has pointed out that in the past the development of speed by animals has often been associated with aridity. Today many desert animals are swift. The ideal invader of the land is not a creeper like *Cenobita*, which, though it can live wholly without gills, drags its protective shell about at night; nor is it such a one as slow, nocturnal, vegetarian *Gecarcinus*, but one like swift, aggressive, more or less diurnal *Ocypode* and tree-climbing *Birgus*. *Ocypode* has lost a third of its gills and developed branchial tufts. *Birgus* has given up its shell and, probably to compensate for the limited use of its abdomen for respiration, has likewise developed branchial tufts in a newly formed compartment of its branchial chamber. Apparently none of the hermits or crabs have hit upon the expedient of turning the skin into the body to make respiratory cavities, such as are represented by the tracheal tubes of certain land isopods, onychophorans, arachnids and insects.

The writer feels that Barrell (1916) and Lull (1917) have perhaps minimized the number of animals which have attained the land directly through the intertidal zone. Judged by the number of species which are today thus partially adjusted to land, there have been many in the past. On the other hand, though many animals live in the estuaries of rivers, very few are able to live in fresh water. Littoral animals perhaps have on the whole met the danger of desiccation as well as that of change in content of body fluids. Land life is attained most easily in the tropics where there is little difference between air and fresh water or ocean temperatures. Pelesneer (1906) has stressed the fact that river faunas developed not only where the temperature was perenially high but also where the ocean was considerably diluted, as in the region of Indo-China where rainfall is very heavy.

Barrell (1916) says "the rarity of the passage of crustaceans, gastropods and vertebrates from truly marine to a truly terrestrial mode of life through the apparently open path of the tidal zone adds to the evidence that an unused food supply could not alone operate as a cause sufficient to induce this change, for so far as the factor is concerned the river faunas would have no clear advantage over those in the tidal zone." The writer feels that such a point of view is perhaps limited and that it directs attention too much to one or another factor as being the primary cause for migration from sea to land. If species are to continue, animals must continually find food, keep from being destroyed by enemies or other dangers in their environment, and reproduce. Food on beaches or in airy fields beyond is always a lure if it is not the subject for undue competition by other species and if its attainment does not seriously interfere with the exigencies of life.

Doubtless, as Barrell maintained, aridity was important as a cause for the evolution of land vertebrates. But aridity is not an universal cause to be applied to all landward migrations. Animals are complex, continually changing systems of activities with complex needs, and they live in complex, changing environments. When they spread into a new situation they must make many compromises between new advantages and new dangers, lost necessities and new abilities. Every habitat offers certain inherent advantages and certain disadvantages. The animals which perhaps best "fit" environment are the homoiotherms, which, because most of their adjusting mechanisms are suited to maintain continual stability within their bodies, can remain continually active, and active at a high level, more or less regardless of ordinary environmental changes.

SUMMARY

(1) At Tortugas certain reef, beach and land animals were studied with particular reference to migrations from sea to land.

(2) Hermit crabs which have become more or less adjusted to life on land show a progressive reduction in the number of gills, and *Cenobita diogenes* lives after its gills have been completely removed.

(3) Crabs which have migrated landward show a progressive lessening of gill-volume. *Ocypode* has lost some gills and developed respiratory tufts on the lining of its branchial cavity.

(4) Beach animals which show any landward trend usually live longer when kept in air than when kept in fresh water.

(5) Animals which have attained some degree of ability to live on land have often also acquired a greater degree of ability to resist the extraction of constituents of body fluids into fresh water. Thus their external membranes tend to maintain stability within their bodies.

(6) Animals which migrate from the sea and become established on land do not do so on account of one "lure" or one "danger." Each habitat has certain advantages and certain disadvantages. A continually changing animal must continually make adjustments to a continually changing environment and, when it migrates to a new habitat, must make many compromises between new advantages and dangers, old necessities and new requirements, and old habits and new abilities.

(7) An ideal land animal is air breathing, water conserving, swift, and internally stable.

(8) *Cenobita* and *Gecarcinus* often have parasitic mites in their gill chambers, but *Ocypode,* which bathes frequently in the ocean, lacks such pests.

TABLE 1—*Showing time in hours which animals lived in sea-water, fresh water, mixtures of sea-water and fresh water, dry glass dishes, and on dry coral sand.*

[The first figure indicates the average for all individuals tested and the second the maximum. + Means that the animal was alive at the end of the observations.]

	No. tested	Sea-water	0.75 salt	0.5 salt	0.25 salt	Fresh water	Air	Sand
Gonodactylus œrstedii Hansen.....	6	624+	12	0.8	0.8	0.4	1.5	
Calcinus sulcatus H. M.-Edwards..	15	334+ 662+	226 597	270+ 662+	23 31	9.5 12	30+*	30+*
Clibanarius tricolor (Gibbes).....	28	485+ 720+	577+ 720+	118 152	20 47	4 4	4.8+*	4.8+*
Cenobita diogenes (Latreille).....	21	51.5 62.8	52.4 64	47.3 50.2	48 52	17 19.2	48 94.5	136 192
Microphis bicornutus Latreille.....	14	123 160	9 9	4.5 6	1.5 2	.5 .5	3.5 6	7 8
Mithrax sculptus (Lamarck).....	28	101 146	6.8 8	5.8 8	.9 1.5	.5 .5	8 8	8 8
Portunus sulcatus (A. Milne-Edwards)	29	198 279	126 252	32 118	.9 .9	.3 .3	.8 .8	.7 .7
Portunus spinimanus Latreille.....	7	12	9	1.5	.5	.5	1	1.5
Callinectes marginatus (A. Milne-Edwards)	7	216+	216+	12+	3	.5	18	20
Leptodius floridanus (Gibbes).....	27	341+ 652+	105 204	106 98	3.8 5	6.3 11.3	6.2 11	6.2 11
Pachygrapsus transversus Gibbes..	21	648+ 648+	648+ 648+	332 450	123 150	1.8 2.2	3.1 4.6	2.5 2.5
Geograpsus lividus (Milne-Edwards)	9	456+	456+	456+	456+	2 2	12 12	12
Gecarcinus lateralis (Freminville); partly in	14	174+ 174+	174+ 174+	169+ 174+	118 161	30 30	85 89	86 91
Ibid.; completely immersed.....	14	32 34	21 21	19 22	12 15	12 12	88+ 96+	96+ 96+
Ocypode albicans Bosc.....	33	307 312+	169+ 312+	291 312+	125 312+	11 18	27 37	19 30
Arca saccharina.....	9	408+	408+	118	217 245	116 144	?	?
Astræa americana Gmelin.....	28	129 142	67 67	48 48	36 48	48 48	56 72	54 60
Strombus gigas Linnæus.....	14	55 58	36 36	30 36	25 25	19 19	36 36	43 50
Cerithium algicola Adams.....	20	432+ 432+	96 96	9? 9?	4? 4?	4? 4?	8? 8?	8? 8?
Nerita versicolor Lamarck.....	28	360+ 360+	140 140	109 140	107 140	96 96	360+ 360+	360+ 360+
Litorina siczac Gmelin.....	28	360+ 360+	159 178	221+ 360+	104 120	97 120	360+ 360+	360+ 360+
Caretta caretta Linnæus 1 week old	7	672+	672+	672+	672+	672+	45	51

* See description of further experiments on p. 213.

	No. of gills	Ratio between volume of body and gills	Branchial tufts	Habitat
Paguristes puncticeps Benedict	26		0	Below low tide
Calcinus sulcatus (H. M.-Edwards)	26		0	Low-tide mark
Clibanarius tricolor (Gibbes)	18		0	Near high-tide mark
Cenobita diogenes (Latreille)	14		0	Land
Callinectes marginatus A. Milne-Edwards	16	21.0:1	0	Pelagic and between tides
Mithrax sculptus (Lamarck)	18	36.0:1	0	Coral reefs
Leptodius floridanus (Gibbes)	18	37.6:1	0	Reefs and alongshore
Plagusia depressa (Fabricius)	18	42.0:1*	0	Rock-walls, low-tide mark
Grapsus grapsus Linnæus	18	34.8:1	0	Rock walls alongshore
Pachygrapsus transversus Gibbes	18	40.0:1	0	Loose rocks and reefs between tides
Geograpsus lividus Milne-Edwards	18	57.0:1	0	Loose rocks, high-tide mark
Gecarcinus lateralis (Freminville)	18	48.1:1	0	Land
Ocypode albicans Bosc	12	61.1:1	5 rows	Sand, beaches and land

* From a specimen which had been preserved in alcohol.

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VII

TWO NEW MITES FROM THE GILLS OF LAND CRABS

By A. S. Pearse

Two text-figures

TWO NEW MITES FROM THE GILLS OF LAND CRABS

During July and August 1928, mites were found at Dry Tortugas on the gills of the land hermit crab, *Cenobita diogenes* (Latreille), and on the Nassau crab, *Gecarcinus lateralis* (Freminville). These crustaceans visit the ocean only once each year when they hatch out their young. No mites were found on the gills of the ghost crab, *Ocypoda albicans* (Bosc), which often visits the ocean and bathes its gills.

Dr. H. E. Ewing has been kind enough to read the manuscript for this paper and grateful acknowledgment is made to him for suggestions. A new genus is also named for him in recognition of his notable contributions to acarology.

EWINGIDÆ, new family

Sarcoptoid mites with tubular pedipalpæ, simple cheliceræ; and pyriform body with few hairs. Legs without suckers and two posterior pair strongly chelate.

Ewingia, new genus

Ewingidæ in which the third and fourth pairs of legs are modified into great forcipate claspers, which resemble those of lice. The pedipalpi are modified into two lateral tubes which enclose the simple cheliceræ. There are no suckers at the tips of the legs. Eyes are absent. Body is pyriform and bears few hairs.

Ewingia cenobitæ Pearse, new species

Host: Cenobita diogenes (Latreille); land hermit crab.

Type Locality: Loggerhead Key, Dry Tortugas, Florida.

Female: (Cat. No. 988, U. S. Nat. Mus.) Average length of the pyriform, smooth body, 0.78 mm.; average width, 0.34 mm. (fig. 1*C*). Length of type specimen, which is unusually large, without capitulum, 0.99 mm.; with capitulum, 1.15 mm. Three pairs of setæ occur on the dorsal surface, the middle pair being lateral and the two other pairs nearer the median line. A pair of small setæ is situated posterior to the anus, which is on the ventral surface near the posterior end. The genital pore is between the coxal plates of the third pair of legs. Capitulum, conical, less than half the length of the remainder of the body; basal segment slightly wider than long. The pedipalpi (*B*) are 2-segmented and form two median surfaces; there are three teeth on each proximal half of the median surface of the distal segment. The cheliceræ (*E*) are 3-segmented, flattened, and linear; the median edge is thin and sharp; there are two teeth at the tip of the distal segment at its median angle.

The two anterioir pairs of legs (*A*) are stout and armed with obtuse strong spines on the three distal segments—four spines on the ultimate segment, and one on each of the others. There are no suckers. The two posterior pairs of legs (*D*) are strongly chelate. The terminal segment of each is tapering, arcuate, and has oblique ridges on its distal inner third. The penultimate segment bears a toothed process on its anterior margin at

about the end of its proximal third; on the lateral surface of this process is a sucker-like area which is surrounded by hairs. The coxæ of the fourth legs are somewhat lateral to those of the third. The bases of the first two pairs of legs are separated from the two posterior pairs.

Larva: Several larvæ (*F, G*) were found on the gills of crabs with the adults. They are a little less than half as long as the adults. There is one pair of chelate legs at the posterior end and two pairs of normal legs some distance in front of these. A pair of stout hairs protrude on either side of the middle of the body and another smaller pair occur on the dorsal surface just behind the capitulum.

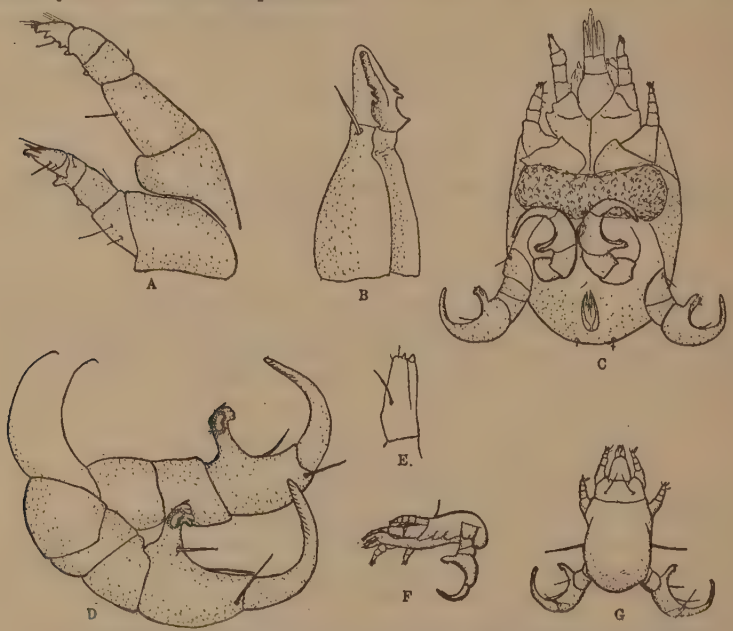

FIG. 1—*Ewingia cenobitæ*, n. sp. *A*, ventral view of first and second legs of right side of female; *B*, pedipalp, female; *C*, ventral view of female; *D*, third and fourth legs of right side, female; *E*, tip of chelicera, female; *F*, side view of larva; *G*, dorsal view of larva.

Eggs: No eggs were observed in the hermit crabs, but several of the females contained eggs in which there were fully formed larvæ which were apparently ready to hatch.

Occurrence: On July 17, 1928, ten specimens of *Cenobita diogenes* from Loggerhead Key were dissected and examined under a binocular microscope. They varied in length from 71 mm. to 19 mm. The number of *Ewingia cenobitæ* on each crab varied from 0 to 172, and the average was 29. On July 18 ten Cenobitas from Garden Key (3 miles from Loggerhead Key) were examined. They varied in length from 75 mm. to 32 mm. None was infested with mites. The mites cling near the outside of the gills with the chelæ on the two posterior pairs of legs, and the body lies between the gill lamellæ.

Family GAMASIDÆ

Lælaps cancer Pearse, new species

Host: Gecarcinus lateralis (Freminville); Nassau crab.

Type Locality: Loggerhead Key, Dry Tortugas, Florida.

Female: (Cat. No. 989, U. S. Nat. Mus.). Body ellipsoidal (fig. 2*A*), slightly truncate posteriorly; length, 1.7 mm.; width, 0.9 mm. About a hundred small setæ are scattered rather uniformly over the dorsal surface. In a terete female the dorsal shield extends from the anterior end to about the posterior sixth of the body. The anterior margin projects forward so as to cover part of the capitulum. The anus is situated at the end of the anterior quarter of an oval area on the ventral surface near the posterior end. The genital opening is in a triangular area between the coxal plates of the fourth pair of legs. The spiracle is dorsal to the coxa of the fourth leg, and the slender peritreme extends forward in a slightly sinuous arc to the mouth parts.

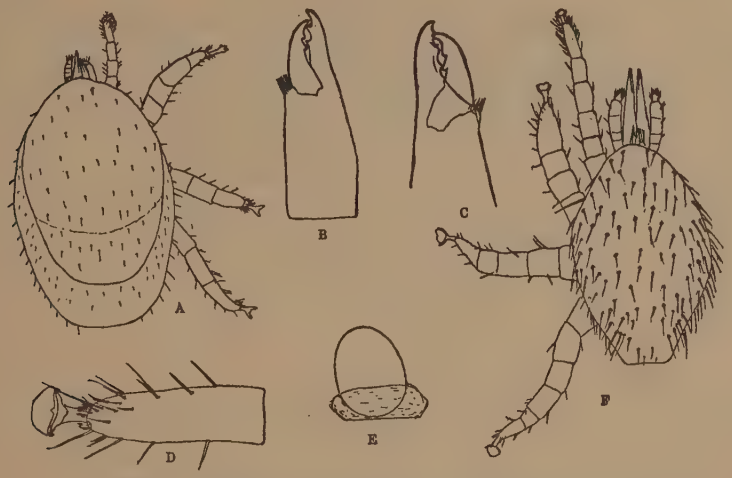

FIG. 2—*Lælaps cancer*, n. sp. *A*, dorsal view of female; *B*, terminal segment of chelicera of female; *C*, tip of chelicera of nymph; *D*, tip of first leg, nymph; *E*, egg attached by cement; *F*, dorsal view of nymph.

The conical, 2-segmented cheliceræ (*B*) are chelate and each finger bears two strong teeth on its inner margin. On the fixed finger there is also a minute tooth just beyond the distal tooth. The pedipalpi are 5-segmented, cylindrical, and setose at the tip. They extend about one-fourth their length beyond the cheliceræ.

The legs are rather slender and terminate in a pad and two spurs (*D*). The tarsal segments are progressively more setose distally in passing from the posterior toward the anterior end of the body.

Nymph: Nymphs (*C, D, F*) of various sizes were found. They are covered dorsally with somewhat stouter setæ than the adults, but in other respects are quite similar to them.

Egg: Eggs (*E*) are commonly found in crabs and are attached in groups along the dorsal, median angles of the branchial chambers by a hardened

secretion. They are plump, ellipsoidal; 0.43 mm. long and and 0.32 mm. wide.

Occurrence: This species was found in the gill chambers of Nassau crabs on Garden Key and Loggerhead Key. One crab examined on July 28, 1928, contained 62 mites, 46 on the right side and 16 on the left. Two individuals examined on August 11 contained 18 and 57 mites respectively. Some of the Nassau crabs were not infested with mites.

Family TYROGLYPHIDÆ

Rhizoglyphus sp.

On August 11, 1928, a single nymphal mite was found on the gills of a Nassau crab, *Gecarcinus lateralis* (Freminville). Dr. H. E. Ewing has examined this and referred it to the genus *Rhizoglyphus*.

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VIII

FURTHER STUDIES ON MARINE BACTERIA WITH SPECIAL REFERENCE TO THE DREW HYPOTHESIS ON $CaCO_3$ PRECIPITATION IN THE SEA

By C. B. Lipman

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FURTHER STUDIES ON MARINE BACTERIA WITH SPECIAL REFERENCE TO THE DREW HYPOTHESIS ON $CaCO_3$ PRECIPITATION IN THE SEA

INTRODUCTION

In 1924 I reported the results of an investigation into the Drew hypothesis of $CaCO_3$ precipitation in the sea through the agency of bacteria. Those results led me to the conclusion that the Drew hypothesis was fallacious or at least unproved. My results and conclusions were based on studies carried out in the South Pacific at the island of Tutuila. It seemed at that time to be desirable to check my results by further studies in one of the regions in which Drew himself worked. I accepted therefore the invitation of the late Dr. A. G. Mayor, Director of the Marine Biological Laboratory of the Carnegie Institution of Washington, at whose instance the original studies were inaugurated, to undertake some additional studies at the Tortugas Laboratory. In the summer of 1922 such new studies were started and completed. A series of circumstances has prevented me from reporting the results obtained until now.

BACTERIAL POPULATION OF SEA-WATER IN THE TORTUGAS REGION

Following the procedure adopted for my studies in the South Pacific I began the Tortugas studies by determining the bacterial numbers in the sea-water at Tortugas both near the beach where the laboratory stands and at distances of a mile to four miles out to sea. The plating in one case was done on a variety of media so as to allow for the demands of as many organisms as possible which make their habitat in the sea, and further to permit of satisfactory comparisons with Drew's results. The results obtained in this first experiment are given in Table I which follows.

TABLE I

Kind of medium	Sea-water 1 mile from shore: number of organisms per c.c. sea-water	Sea-water 4 miles from shore: number of organisms per c.c. sea-water	Sea-water near beach: number of organisms per c.c. sea-water
Gran's medium.....	20	..	.....
Gran's medium + KNO_3.....	...	..	.....
Drew's peptone agar.	150	10	400,000
Sea-water agar.....	30	..	.....
Calcium lactate agar.	73	..	.....
Calcium acetate agar.	20	..	.....

The descriptions of the media named in Table I are given in the appendix to this paper. It is clear from the foregoing data that even

within relatively narrow limits the composition of the medium is an important determinant of the numbers of organisms found in sea-water as in other substances, the number being 7.5 times as large on Drew's peptone agar as on either Gran's medium or on calcium acetate agar. Sea-water agar which contains nothing but sea-water and agar is a medium which, so far as the writer is aware, has never been used by anyone but himself. This is a curious circumstance because the medium is one which should naturally suggest itself to the investigator and because there is much to be learned from its use. Further reference to this medium will be made below. For the present we need merely note that as regards bacterial counts on sea-water agar it is not so inferior a medium as one might at first suppose. Turning now to the more important features of the foregoing data we see first that the bacterial population of sea-water diminishes very rapidly as we go farther out to sea. While this fact has been reported by the writer and by others much earlier, the aggregate of evidence for it has not been very great. It should be pointed out next that the data for counts which are given in Table I represent platings made within two hours after the water samples were collected. If the samples are allowed to stand even twelve hours the counts rise approximately, and after a few days standing will rise very markedly. This was determined by numerous platings. Furthermore, counts made from different samples even one day apart at the same place may vary markedly. The chief thing to note in these data, however, is the paucity in bacterial numbers in pure surface sea-water. While, as I have just observed, this number may vary considerably from day to day or week to week, I have never found the absolute number of bacteria near Tortugas and out in the uncontaminated open sea to exceed 200 per cubic centimeter of sea-water. I stress this point because of its important bearing on the principal theme of this paper which is discussed below. When compared with similar conditions in the South Pacfic the sea-water of the South Atlantic is somewhat poorer in bacterial population, but not much.

ISOLATION AND STUDY OF SEA-WATER BACTERIA

Sea-water from near the laboratory dock and that from out in the Gulf Stream at a distance of four miles from the island were plated on Drew's peptone agar and on other media described in the appendix. By a careful examination of all the colonies which developed, it was possible to isolate a large number of organisms which represent perhaps most of the different forms to be encountered in surface sea-water. Each type of organism thus isolated was inoculated into a great variety of media which were compounded so as to determine the power, if any, of these organisms to precipitate $CaCO_3$.

TEST OF DIFFERENT ORGANISMS ISOLATED FROM SEA-WATER IN $CaCO_3$ PRECIPITATING POWER IN DIFFERENT MEDIA

In my investigations in the south Pacific the pure cultures of bacteria isolated from sea-water were shown to be possessed, with rare exceptions, of the power to precipitate $CaCO_3$ in appropriate media. In that case, however, isolations were made from sea-water which was only slightly contaminated by drainage from the shores of Pago Pago Bay. On the other hand, as indicated above, many more organisms were isolated at Tortugas than at Pago Pago, owing to the fact that sea-water for the isolations was collected from a spot close to the shore. Under these circumstances one would expect to obtain pure cultures of a considerable number of bacterial forms which are foreign elements in the flora of sea-water and therefore, unlike most of the normal inhabitants of that medium, unable to precipitate $CaCO_3$, even in appropriate media. It seemed desirable, nevertheless, to test all of these organisms in a great variety of media in order to determine how large a proportion of them possess the power of precipitating $CaCO_3$, and in addition to learn which of the constituents of the media determine their suitability to the phenomenon of the precipitation of $CaCO_3$. The results of these experiments follow. In each case the numbers attaching to the organisms and to the media refer to descriptions of them given in the appendices to this paper.

TABLE 2—*Bacteria in Tortugas sea-water capable and incapable of precipitating $CaCO_3$ in various media*

Medium number	Number of organisms capable of precipitating $CaCO_3$	Number of organisms not capable of precipitating $CaCO_3$
68	None	14, 19, 21, 24, 25
69	29	15, 17, 18, 23, 26, 29
59	29	14, 15, 18, 19, 21, 23, 24, 25, 26, 27, 30
15	Little or no growth	Little or no growth
17	Little or no growth	Little or no growth
53	25, 29	14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 26, 27, 28, 30, 31
64	Very little growth	Very little growth
63	Very little growth	Very little growth
20	None	14, 15, 18, 19, 21, 23, 24, 25, 27, 29, 30
16	None	14, 15, 18, 19, 21, 23, 24, 25, 26, 27, 29, 30
54	29, slight precipitation	14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31
58	14, 29	15, 18, 19, 21, 23, 24, 25, 26, 27, 30
19	No Growth	No growth
18	None	14, 15, 18, 19, 21, 23, 24, 25, 26, 27, 29, 30
52	18, 19, 21, 23, 24, 29	14, 15, 16, 17, 20, 22, 25, 26, 27, 28, 30, 31
67	None	14, 15, 18, 19, 21, 23, 24, 25, 26, 27, 29, 30

It will be noted in the foregoing table that in all but two cases precipitation of $CaCO_3$ was obtained only in media in which large amounts of soluble calcium salts had been included. In the exceptional cases mentioned,

impurities in the organic compounds used were probably responsible for the results obtained as indicated below. It is highly interesting to note how many organisms are capable of precipitating $CaCO_3$ if a proper medium is supplied, and at the same time how much better adapted to the purpose than all the other organisms tested is the one I have numbered 29. These data would seem to furnish further support for the idea advanced by me in earlier papers on the non-specificity, in the strict sense, of organisms concerned with calcium precipitation in the sea. The behavior of organisms in medium 52 shows that, given the right medium, many bacteria may adapt themselves to the task of $CaCO_3$ precipitation, but that without such a medium even the most effective calcium precipitating organism found is without power to bring about the precipitation. My results also make clear that neither organic matter nor KNO_3 is the crucial constituent of media intended to show $CaCO_3$ precipitation, but that a considerable excess of soluble calcium is essential. This also bears out the results of another experiment which is described below. It should be added that while $CaCO_3$ precipitation in these cultures was always an accompaniment of good growth, the latter was frequently obtained in cultures without a trace of $CaCO_3$ precipitation.

DOES ORGANIC MATTER IN SEA-WATER INFLUENCE THE PRECIPITATION OF $CaCO_3$ BY MARINE BACTERIA?

Some of the proponents of the hypothesis that certain marine bacteria possess the power of precipitating $CaCO_3$ in sea-water, while admitting that such bacteria can not carry out such precipitation in ordinary sea-water as proved by the author in earlier publications, insisted that they can do so in sea-water carrying organic matter in solution. An experiment was therefore arranged to test this point as follows: Large amounts of the sea-weed *Sargassum*, which occurs commonly in the sea in the Tortugas region, were crushed in sea-water and the masses subjected to heating in the autoclave. The solution thus prepared was filtered and distributed in a large number of one-gallon glass museum jars, covered with cottom tops and sterilized. The solutions in the jars were then inoculated with marine bacteria; some with pure cultures as described above, others with mixed cultures, and still others with fresh sea-water. These were all incubated in the laboratory for two weeks and then examined for evidences of $CaCO_3$ precipitation. In no case did I secure any evidence that $CaCO_3$ is precipitated under such conditions, either by pure cultures or by mixed cultures of marine bacteria.

These results seem to render it in the highest degree improbable that such organic matter as might be present in sea-water could produce conditions favorable to the precipitation of $CaCO_3$ from sea-water. While organic matter of animal origin was not employed in these experiments it

seems hardly likely that it could be any more effective than that of plant origin like *Sargassum*.

PRECIPITATION OF $CaCO_3$ IN PEPTONE MEDIA

In earlier communications I pointed out that my experiments had led me to the conclusion that precipitation of $CaCO_3$ in sea-water could only be effected by marine bacteria when soluble calcium compounds are added to the sea-water; and therefore that calcium naturally occurring in sea-water could not be precipitated as calcium carbonate by the marine bacteria. An apparent contradiction to this conclusion was offered in the fact that $CaCO_3$ was precipitated by marine bacteria in cultures made up merely of sea-water and peptone. At first puzzled by this fact I began to consider the possible cause of this exceptional phenomenon when it occurred to me that peptone might contain soluble calcium compounds. In order to test this surmise I shook up small and large amounts of peptone with pure distilled water, filtered the solutions and tested for soluble calcium with ammonium oxalate. In every case tested, large quantities of soluble calcium were found in peptone. The apparent contradiction to my earlier conclusions was thus dispelled and no exception now exists to the conclusion which I formulated, that marine bacteria do not possess the power of precipitating $CaCO_3$ from sea-water unless there be added to the sea-water soluble salts of calcium in addition to those naturally occurring there, and only then if such additions are considerable.

PRECIPITATION OF $CaCO_3$ IN SEA-WATER WHEN INORGANIC SALTS OF CALCIUM ARE ADDED

In my report on the Samoan investigations on $CaCO_3$ precipitation in sea-water I proposed, among others, an explanation for such precipitation based entirely on a change in chemical equilibrium among the components of the sea-water. In that I pointed out that any change in the equilibrium which results in the relative piling up of the Ca ion must be accompanied by the precipitation of calcium salts. At Tortugas I arranged an experiment to test this idea. Two series of solutions were prepared with sea-water as a basis, as follows: One was composed of sea-water to which was added 0.1 per cent KNO_3 and 0.2 per cent $CaCl_2$. The other was composed of sea-water to which was added 0.1 per cent KNO_3 and 0.2 per cent $CaSO_4$. These solutions were distributed in small flasks, some of which were sterilized, whereas the others remained unsterilized but similarly cotton stoppered. In a few days all the flasks were examined and all were found to contain precipitated $CaCO_3$ in both the sterile series and the unsterilized series, except that the solutions to which $CaCl_2$ had been added had larger precipitates than those to which $CaSO_4$ had been added. This circumstance may probably be accounted for entirely by the larger amounts of calcium added in the first salt as compared with those added in the sec-

ond salt. But in both cases it is clear that bacteria play no part in at least one way of causing precipitation of $CaCO_3$ in sea-water.

PRECIPITATION OF $CaCO_3$ BY AN INCREASE IN THE HYDROXYL ION CONCENTRATION OF SEA-WATER

Another proposal which I made in my report on the Samoan investigations was that a change in chemical equilibrium induced in sea-water by the addition of hydroxyl ion must result in the precipitation of calcium salts, which precipitates may later be found to contain $CaCO_3$. As an experimental check on this proposal I carried out the following experiment. To each of two small flasks containing sea-water, enough NH_4OH was added to cause precipitates to be formed. The precipitate in one of the flasks was examined immediately and was found to contain calcium salts, but no $CaCO_3$. The other flask was allowed to stand for several days, after which its precipitate was examined and found to contain large amounts of $CaCO_3$.

In both this experiment and in the one described in the foregoing section it is clear that $CaCO_3$ precipitation can be produced directly or indirectly by bringing about a new equilibrium among the constituents of the sea-water. In nature, such phenomena doubtless occur through the introduction of added quantities of Ca by drainage into the sea or by decay of organic matter which sets Ca free from its combinations in the first instance given above, and by the increase of hydroxyl ion concentration through intense photosynthesis by marine organisms in the second instance given.

A STUDY OF THE CALCAREOUS MUD DEPOSITS FROM THE VIEWPOINT OF THE DREW HYPOTHESIS

It has been contended by the advocates of the Drew hypothesis that, while it may not be true that precipitation of $CaCO_3$ occurs in sea-water as such, it might be expected to go on at a rapid rate in muds in contact with sea-water. It has been argued that the mud would carry much larger populations of $CaCO_3$ precipitating bacteria than sea-water and therefore might be responsible for the phenomenon of precipitation on a large and significant scale. In order to test this idea I subjected the mud from near Fort Jefferson to a study for bacterial population. The samples of mud used for the purpose were furnished by Dr. W. H. Longley who secured them from a mud hole by diving at a depth of about eight fathoms from the surface of the sea and from a location close to that from which Drew obtained his mud samples for study. The samples were collected in sterile containers and were plated immediately after their arrival at the laboratory. The plating was carried out with the wet mud, the water content being determined upon a separate sample, so that the counts could be given per gram of dry mud. The medium described in the appendix as No. 25 was used. A statement of the counts obtained after incubation follows:

Number of bacteria in mud from near Fort Jefferson

	No. per gram dry mud
After 40 hours	10,000
After 64 hours	22,000
After 4 days	22,000

The sea-water above the mud showed a count of about 150 colonies per cubic centimeter of sea-water. About 30 per cent of the colonies appearing on the plates were deep, somewhat shadowy colonies with a distinct central nucleus.

It can be seen from the figures given above for the bacterial counts on the mud, that the population thereof is decidedly small and one which can hardly be considered adequate for the task of $CaCO_3$ precipitation on a significant scale. Some more powerful agent than such a calcium precipitating flora as that characterizing the mud would be required to account for the laying down of immense masses of calcareous material in the Bahama mud banks and in similar places. It would seem therefore that there is little more evidence in the mud than in the sea-water in support of the hypothesis promulgated by Drew relative to the manner of $CaCO_3$ precipitation in the sea.

CONCENTRATION OF THE CONSTITUENTS OF A SUITABLE MEDIUM FOR $CaCO_3$ PRECIPITATION BY BACTERIA AS A VITAL FACTOR IN THE PHENOMENON

In this and in earlier papers I have emphasized repeatedly the fact that I have been unable to detect any precipitation of $CaCO_3$ by marine bacteria in media not containing a considerable excess of soluble calcium salts over and above the normal amount in sea-water. In support of this I have furnished evidence in earlier papers and confirmed by new evidence cited above, that I have never observed the precipitation of $CaCO_3$ in normal sea-water inoculated with mixed or with pure cultures of marine bacteria of any kind. It seemed desirable in addition to strengthen these findings by similar tests with media made up qualitatively like the most favorable medium for $CaCO_3$ precipitation by marine bacteria but differing quantitatively from it. Accordingly the following medium was made up, sterilized and inoculated in different portions with pure and with mixed cultures of marine bacteria.

Composition of dilute calcium lactate medium

Calcium lactate	0.50 grams
Peptone	.25
KNO_3	.05
Na_2HPO_4	.05
Filtered sea-water	1,000

Twelve pure cultures isolated from sea-water and sea-water itself were tested with this medium. Some cultures yielded slight growth therein, some

fair growth, and some good growth, the best growth being obtained in the mixed culture, but in no case was there any evidence of the precipitation of $CaCO_3$ by the marine bacteria. This confirms the claims which I have repeatedly made to the effect that for precipitation of $CaCO_3$ from sea-water by marine bacteria the normal supply of calcium is inadequate and that even moderate additions of calcium lactate to sea-water do not result in fitting sea-water as a medium from which $CaCO_3$ may be precipitated by the bacteria in question.

IS THE NITRATE ION ESSENTIAL IN MEDIA SUITABLE FOR PRECIPITATION OF $CaCO_3$ BY MARINE BACTERIA?

Incidental to my experimental inquiries at Tortugas came the revelation that the addition of the nitrate ion to sea-water is unnecessary in addition to calcium salts and other constituents described in medium No. 25 and similar media to insure the precipitation of $CaCO_3$ by marine bacteria as has been claimed by Kellerman and Smith[1] and by Drew.[2] Gran's medium, inclusive and exclusive of KNO_3, was used in this experiment. This was inoculated in some portions with sea-water and in other portions with pure cultures isolated as described elsewhere in this paper. In all cultures $CaCO_3$ was found as a crystalline precipitate after the requisite period of incubation. This proves that the essential substance required for addition to sea-water media in order to insure precipitation of $CaCO_3$ is some soluble calcium salt and not KNO_3. It shows, moreover, that Drew's idea relative to the essential denitrifying function of calcium precipitating bacteria is fallacious and that the nitrate content of such media as those in question is a factor of purely incidental value.

PRESENT STATUS OF THE DREW HYPOTHESIS

Taken together with the results and discussions furnished in my earlier papers, the evidence given above leads one to deny emphatically the tenability of the hypothesis put forward by Drew, supported by Kellerman and Smith and adopted by geologists fairly generally as the explanation of the manner of precipitation of $CaCO_3$ in the calcareous mud banks and in similar deposits. Curiously enough the evidence which I furnished in 1924 has apparently escaped the attention of at least one other student[3] of the subject who has published relatively recently a paper on calcium precipitating organisms on which I desire to comment critically. The author to whom I refer is the distinguished German physiologist Hans Molisch. In 1925 that investigator described two organisms, regarded by him as new, which possess the power of precipitating $CaCO_3$, one in sea-

[1] Jour. Wash Acad. Sci., vol. 4, No. 14, 400 (1914).

[2] Report Department Marine Biology, Carnegie Inst. Wash. Year Book No. 10, 136-141 (1911), and No. 11, 136-144 (1912). See also Papers from the Tortugas Laboratory, Carnegie Inst. Wash., vol. 5, 9-45.

[3] Cent. Bakt. 2te Abt., vol. 65, 130. 1925.

water and one in fresh water. Molisch was apparently unaware of the work of any other investigators in the field when he carried out his experiments during a visit in Japan. Before writing up his experiments he did discover the papers of Kellerman and Smith and of Drew, after which he claims to have arrived independently at the same conclusions as those authors. Surprisingly, however, he gives a new name to the calcium precipitating organism isolated by him from sea-water—*Pseudomonas calciprœcipitans*. About this organism he says:

"Diese Bakterie ist nicht *Pseudomonas calcis* sondern eine andere neue *Pseudomonas*-Art, der ich den Namen *Pseudomonas calciprœcipitans* gegeben habe. Verglichen mit der *Ps. calcis* die leider nur sehr unvollkommen beschrieben ist, ist meine Bakterie viel schmäler und durchschnittlich etwas länger."

Anyone fully acquainted with the precariousness of establishing new bacterial species on morphological grounds must view with astonishment Molisch's attempt to name a new species on the slender differences between his organism and that of Kellerman and Smith which are given in Molisch's own words above. Moreover in the face of evidence on the non-specificity of the phenomenon of bacterial precipitation of $CaCO_3$ and the non-existence of the phenomenon in sea-water unfortified by large amounts of soluble calcium salts which I furnished in my earlier papers (and confirmed in this one) it is amazing to note the appearance of Molisch's statements. Another statement made by Molisch deserves critical comment. He says:

"Nach meinen Untersuchungen ist es nicht notwendig, der Nährlösungen, wenn sie mit Meerwasser gemacht werden, noch ein Kalksalz zuzusetzen, der Gehalt des Meerwassers an Kalk, obwohl er nicht sehr grosz ist, genügt für die Fällung schon allein. Ich habe aber beobachtet, dasz ein Zusatz eines Kalksalzes sei es eines organischen wie essigsauren Kalkes oder eines anorganischen wie $CaCl_2$ die Fällung des CO_3Ca durch die Bakterie beschleunigt und begünstigt."

The second sentence in the foregoing quotation requires little comment since other investigators and I have shown it to be true that addition of calcium salts to the proper media insure marked precipitation of $CaCO_3$ by marine bacteria, but I have proved in this paper that such addition of calcium salts not only furthers the phenomenon of $CaCO_3$ precipitation but that it is essential to the very occurrence thereof. This leads me to comment on the first sentence in the quotation. Molisch is seemingly unaware of the fact of which I have furnished proof above, that soluble calcium salts may be furnished to a medium without adding them as such. I have shown that peptone contains large quantities of soluble calcium. Molisch uses large quantities of peptone in his media without suspecting that he is adding any calcium to his sea-water media. The following is the composition of the medium which he employed: 1,000 grams sea-water, 10 grams peptone, 5 grams glycerine. This medium may seem therefore innocent of

calcium content except that in sea-water, but we can now see clearly the error of Molisch's conclusion with respect thereto.

Had Molisch appreciated the significance of the results cited by him in the same communication as obtained with fresh-water media he would doubtless have refrained from making the statement appearing in the earlier part of his paper, and also from his attempt to establish a new species of organism in fresh water which is capable of precipitating $CaCO_3$ as well as the one which he described from sea-water. The fact that he describes bacteria, yeasts, molds and actinomyces species concerned with $CaCO_3$ precipitation should have signified something much more to him relative to the whole problem than it did.

From my experiments at Samoa in 1920 and those at Tortugas in 1922, it seems proved beyond peradventure that calcium precipitation in sea-water is a phenomenon which under the general conditions of nature is referable to physical-chemical conditions which characterize the oceans, and under their influence a number of reactions may occur which result in the deposition of $CaCO_3$. What such reactions may be I have pointed out in one of my earlier papers.[1] More recently Irving has amplified and added to the discussions of one or two of the points made in my paper.[2] That calcium carbonate may be precipitated in these ways there can be no question. Whether or not such a phenomenon may occur in addition under the conditions existing in nature and on a vast scale through the agency of bacteria is a question which I have answered in the negative or at least have clearly shown that proof is lacking in support of such a view. Much the same views may be expressed relative to precipitation of $CaCO_3$ in fresh water.

In connection with these general considerations on the subject under discussion it must be emphasized again that the paucity in bacterial population in the uncontaminated stretches of the oceans is another vital bit of evidence against the Drew hypothesis and the idea that $CaCO_3$ might be precipitated by bacteria on any significant scale in the sea.

SUMMARY AND CONCLUSIONS

1. Bacterial populations in the open sea are very small, scarcely ever showing more than 150 organisms per cubic centimeter of sea-water. Usually the numbers are much smaller.

2. Mixed or pure cultures of organisms isolated from sea-water are incapable of precipitating $CaCO_3$ from sea-water to which no salts have been added, or even in the presence of added KNO_3.

3. Mixed or pure cultures of such organisms are incapable of precipitating $CaCO_3$ in a sea-water medium if KNO_3 and organic matter as the sugars or similar forms free from calcium are added to the medium.

[1] Papers from the Department of Marine Biology, Carnegie Inst. Wash., vol. XIX, 179–191, 1924.

[2] Jour. Mar. Biol. Assoc. United Kingdom, vol. 14, 441–446. Aug. 1926.

4. Such organisms must be supplied in addition to sea-water with some soluble calcium salt, organic or inorganic, if they are to be proved capable of precipitating $CaCO_3$.

5. A number of different forms of bacteria in the sea possess the power of precipitating $CaCO_3$ in appropriate media containing a large excess of soluble salts, but only under such conditions. Among such organisms there is great variability to perform the task in question, depending on the composition of the medium.

6. The requisite soluble calcium salts may be furnished not only by inorganic and organic salts of calcium, but also by peptone, proteins and similar preparations, which I have shown to contain large quantities of soluble calcium salts.

7. KNO_3 is not needed for $CaCO_3$ precipitation under sea-water conditions as was claimed by Kellerman and Smith.

8. The errors into which Molisch was led in his paper on calcium precipitating bacteria are pointed out and the reasons given for his having been led into them. Other features of his paper are discussed.

9. Upon the basis of evidence in this and in my earlier papers the Drew hypothesis is shown to be untenable, and at the very least unproved. The strong probability is reemphasized of the purely physical-chemical nature of $CaCO_3$ precipitation on a large scale in nature.

I desire to express my thanks for the faithful and valuable assistance rendered me in these studies by Dr. J. L. Starkey of the New Jersey Agricultural Experiment Station. It is also a pleasant duty to acknowledge my obligation to the Carnegie Institution of Washington which made the studies possible.

Appendix I—DESCRIPTION OF MEDIA USED IN STUDY OF MARINE BACTERIA

1. Gran's medium.
2. Gran's medium minus potassium nitrate.
3. 1 per cent peptone in sea-water.
4. 1 gram calcium lactate, 1 gram peptone, 0.5 gram potassium nitrate, 1,000 c.c. sea-water.
5. Drew's peptone agar.
6. 1 gram calcium acetate, 1 gram peptone, 0.5 gram potassium nitrate, 1,000 c.c. sea-water.
7. 1,000 c.c. sea-water, 15 grams agar.
10. Same as No. 4.
11. Same as No. 4 but in place of pure sea-water, 0.75 sea-water and 0.25 distilled water.
12. Same as No. 10 but in place of pure sea-water, 0.5 sea-water and 0.5 distilled water.
13. Same as No. 10 but in place of pure sea-water, 0.25 sea-water and 0.75 distilled water.
15. 2.5 grams saccharose, 0.1 gram disodium-hydrogen-phosphate, and 500 c.c. sea-water.
16. Same as No. 15 but with 0.05 gram KNO_3 added.
17. 2.5 grams dextrose, 0.1 gram disodium-hydrogen-phosphate, and 500 c.c. sea-water.
18. Same as No. 17 but with 0.05 gram KNO_3 added.
19. 2.5 grams lactose, 0.1 gram disodium-hydrogen-phosphate, and 500 c.c. sea-water.
20. Same as No. 19 but with 0.05 gram KNO_3 added.
21. Calcium lactate 5 grams, potassium nitrate 2 grams, disodium-hydrogen-phosphate 0.25 gram, sea-water 1,000 c.c.
30. Extract of *Sargassum*, made by boiling one part of *Sargassum* in 2 parts sea-water and filtering.
31. Same as No. 30 but with 0.05 gram KNO_3 added.
32. Soil extract, made by boiling calcareous soil of Tortugas Keys with sea-water; 1 part of soil to 2 parts of sea-water.
33. Same as No. 32 but with 0.05 gram KNO_3 added.
50. 2 grams sodium malate, 1 gram peptone, 0.5 gram potassium nitrate, 1,000 c.c. sea-water.
51. Same as No. 50 but acidity of sodium malate neutralized with potassium hydrate.
52. 5 grams calcium lactate, 2 grams potassium nitrate, 1 gram peptone, 0.25 gram disodium-hydrogen-phosphate, 1,000 c.c. sea-water.
53. Same as No. 52 but with calcium citrate substituted for calcium lactate.
54. Same as No. 52 but with calcium succinate substituted for calcium lactate.
58. Same as No. 52 but with sodium lactate substituted for calcium lactate.
59. Same as No. 52 but with sodium citrate substituted for calcium lactate.
63. *Sargassum* after rotting extracted with sea-water; 1 part *Sargassum*, 2 parts sea-water.
64. Same as No. 63 but with 0.05 gram KNO_3 added.
66. 100 c.c. soil extract, 900 c.c. sea-water.
67. 0.5 gram calcium lactate, 0.25 gram peptone, 0.05 gram potassium nitrate, 0.5 gram disodium-hydrogen-phosphate, 1,000 c.c. filtered sea-water.

Appendix II—ORGANISMS ISOLATED FROM SEA-WATER PLATED ON DIFFERENT MEDIA

1. Most common colony occurring. Spreading, translucent, deep colony, usually with tiny opaque nucleus. Short rod or *Pseudomonas;* stains solidly but lightly with methylene blue. Usually occurs singly, sometimes in pairs; commonly stained polar granules are visible.
2. Small, raised, opaque, viscid colony, creamy white. Micrococcus; stains deeply with gentian violet. Occurs as single cells.
3. Raised, opaque, viscid colony, medium size, creamy white. Short rod, about half as long again as No. 1; no *Pseudomonas* forms visible. Commonly occurring in pairs.
4. Medium size, irregular shaped, raised, viscid, surface colony, creamy white. Short rod much like No. 1 but longer and occurs commonly in pairs; stains deeply with gentian violet.
5. Tiny, white, opaque, surface colony. Cells elliptical; possibly *Pseudomonas,* but larger than No. 1 and has a refractive, non-staining central body resembling a spore; otherwise stains deeply. Typical rods almost absent.
6. Deep colony, medium size, translucent. Common on all plates. Something like No. 1 but more perfectly a *Pseudomonas* form. Very likely the organism described by Drew.
7. Like No. 6 but very small colony; cells like No. 6.
8. Very tiny colony, otherwise like those of Nos. 1, 6 and 7. Short rod, but greatly variable as regards length. *Pseudomonas* forms rare.
9. Yellowish red, very small, deep colony. Cells in long tangled threads or slender filaments; apparently cells in sheaths. Probably an actinomyces form.
10. Large colony, but otherwise like No. 1. Cells closely similar to No. 1.
11. Very large, commonly occurring colony. Cells like No. 1.
12. Perfectly circular, shadowy colony. Organism like No. 9, but the threads are few and short.
13. Somewhat raised, small, surface, creamy-white colony. Probably identical with No. 5.
14. Large, raised, apparently striated from center as from a spoke, markedly mucilaginous colony. Cells appear as large rods, somewhat thicker but shorter than *B. subtilis.*
15. Large surface colony with raised central nipple. Cells occur as coccus forms. Probably identical with No. 24, described below.
16. Tiny, lenticular, opaque, dense, deep colony. Cells occur as slender rods five or six times as long as they are wide. No evidence of *Pseudomonas* forms, but organism is very variable. Stains solidly with gentian violet.
17. Tiny, deep red, lenticular colony. Cells occur as slender rods showing tendency to form filaments. Probably identical with No. 9 and possibly 12 and 16.
18. Apparently but not certainly one of the commonest forms occurring in sea-water. Short rods and coccus forms, the latter tending to be elliptical.

19. Deep colony forming in shape of a four-leaved clover. Cells occur as *Pseudomonas* forms, accompanied by what appear to be short fat rods; marked polar granule staining, light area between granules. The most common of the marine bacteria precipitating $CaCO_3$.
20. Small, circular, surface colony, red-brown center; probably surface form of one of the deep colonies described. While much smaller than No. 19 and staining less deeply it shows many similarities to it.
21. Almost circular, dense, very small, deeply opaque, white colony. Short rod, staining uniformly; commonly occurring in pairs.
22. Very small, pink colony, dense, deep, making funnel-like depression in agar. Elliptical cells or small coccus forms enclosed in sheaths or occurring in chains or filaments. Apparently like No. 9. Organism has solvent powers for calcium phosphate precipitated in the medium, thus forming halo around the colony.
23. Apparently like No. 22 in appearance as regards the colony. Cells as small coccus forms. Probably but not certainly identical with No. 22.
24. Medium size, red, surface colony with raised central nipple. Cells occur as micrococci; stain solidly with gentian violet. Probably identical with No. 15.
25. Large, spreading, deep, translucent colony, lace-like in texture. Cells as short rods in pairs; stain deeply and solidly; rods appearing closely similar to No. 21.
26. Reddish, medium-size colony, deep, making a halo and a depression in the agar; something similar to No. 22 and No. 23. Long, filamentous, slender rods, some like No. 9, some like No. 22.
27. Beautiful, large, well-raised, circular, vitreous to opaque colony like one described in paper on Samoan studies cited above. Central large nipple, surrounded by a groove, then a rise with striations. Markedly mucilaginous, large, elliptical cells; walls staining deeply, interior solidly but lightly stained. Much larger than *Pseudomonas* forms above described. In some ways resembles Azotobacter. Makes especially good growth in lactate media. Not numerous in Tortugas sea-water but numerous in Samoan sea-water.
28. Leaf-like surface colony, creamy white. Very short rod; stains lightly.
29. Circular, creamy-white, surface colony. Cells appear identical with No. 28.
30. Very large colony; otherwise surface appearance something like No. 29. Very short, slender rods occurring largely in pairs and then seeming like comma bacillus; stains lightly. In spite of appearance of colony it does not seem to be identical with No. 1. There is some tendency shown to the *Pseudomonas* form.
31. Medium-size colony, dense, deep. Small elliptical cells in long ragged chains or sheaths; sometimes showing deeply stained polar granules. Resembles No. 9; probably identical therewith.

100. Small, lenticular, surface colony, yellowish white but red in center. Cells like No. 1.
101. Medium-size, surface colony. Yellowish red with decided raised nipple in center. Otherwise apparently identical with No. 100.
102. Small, circular, raised, creamy-white colony with red or pink center. Probably the same as the foregoing.
103. Smaller and flatter but much deeper, red, surface colony than No. 102. Cells, however, similar.
104. Small, deep, semi-transparent colony with opaque dot for a central nucleus. The cells appear similar to No. 1. Probably the most abundant colony on sea-water plates at Tortugas.
105. Same as No. 102. More pearly in appearance.
109. Lenticular, medium-size colony, raised from surface, pink in center, white rim. Probably the dominant type of colony.
110. From pink to reddish, circular, raised, viscid colony. *Pseudomonas* form.
111. Colonies twice the size of No. 110. Raised, circular, reddish-brown colony with central raised nipple. Cells similar to No. 110.
112. Raised, large, mucilaginous colony, erose edges, probably striated from center. Resembles and probably is identical with No. 27.
113. Circular, pink colony. Cells similar to No. 112.
114. Very large, striated, circular colony. Probably identical with No. 27.
115. Large, opaque, viscid, mucilaginous colony, circular; tiny nipple in center of small funnel-like depression. Cells similar to No. 114.
116. Thin surface colony, decidedly serrated, at periphery; nucleus in center. Short rods staining solidly.
117. Watery, transparent colony at surface of medium. Organism occurs very commonly. Tiny, deep nucleus at center of colony, but otherwise growth transparent. Very short rods.
118. White, with reddish-brown central nipple. Medium size, elliptical cells, staining solidly.
119. Pink colony, medium size, deeper in color at center, almost pearly in appearance. Cells as *Pseudomonas* and short rod forms of about similar size. Probably the most efficient organism for precipitating $CaCO_3$ from artificial media.
120. Isolated from deep mud near Fort Jefferson. Large, spreading, opaque colony; central dot for nucleus; serrated edges. One of the very common organisms in the sea-water and muds about Tortugas. Colonies always much larger than those made up of *Pseudomonas* forms. Very slightly raised above surface. No pigment or halo produced. Probably identical with No. 116 but not certainly. Frequently forms giant colonies, at times spreading over the whole plate.

121. Large, deep colony. Probably identical with No. 102.

122. Large, transparent, watery colony. Probably identical with No. 117 but larger and with a circular rim and with good size nucleus in center. Short rods.

123. }
124. } Probably identical with No. 109.

IX

THE CHEMICAL COMPOSITION OF SEA-WATER

By C. B. Lipman

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THE CHEMICAL COMPOSITION OF SEA-WATER

Many studes of marine biology can not proceed beyond superficialities, without a more or less detailed knowledge of the composition of sea-water. Unfortunately, such knowledge has not been and is not now available. While it is realized that it may never be practicable to obtain most thorough and complete analyses of the inorganic constituents of sea-water, it is feasible to obtain much more information of that sort than has been published to date. The many partial analyses which have been published have been concerned with only a few of the ionic constituents, usually calcium, magnesium, potassium, sodium and chlorine. In his studies on the marine bacteria and related subjects, the author came early to feel the need of more accurate and complete analytical data on the inorganic components of sea-water and determined to obtain them. Through the interest of the late Dr. A. G. Mayor I have been enabled to carry on a certain amount of analytical work on sea-water which I regarded as a substantial contribution toward the desired end. The analyses in question having now been completed, I am enabled to render the following report with respect to them.

SAMPLES COLLECTED FOR STUDY

Circumstances rendered it convenient to collect a preponderance of samples for this study from the Pacific area. In fact, only two samples were analyzed from the Atlantic area and they were both from the Gulf Stream, and taken near Loggerhead Key in the Tortugas. The latter were collected about three months apart in the summer of 1922. While thus most of the samples analyzed were taken in the Pacific region, the samples from the latter were widely scattered in that area and represent tropical, as well as temperate, zones. The samples collected represent surface sea-water only, at no depth greater than one meter. It is important to make note of this in view of the marked changes in composition which accompany the descent into the deeper water of the ocean, as shown by analyses for a few constituents made recently by Atkins and Harvey.[1] The following is a description of the several samples of sea-water analyzed:

1. Gulf Stream one mile from Loggerhead Key, Dry Tortugas. Collected July 1922. Analyzed by A. L. Sommer.
2. Pacific Ocean, off Astoria, Oregon (No. 14). Analyzed by Muriel Ashley.
3. Pacific Ocean, off Astoria, Oregon (15). Analyzed by Muriel Ashley.
4. Pago Pago Bay, at Aua Reef. Collected July 1920. Analyzed by D. M. Greenberg.

[1] Nature, vol. 116, 784.

5. Pacific Ocean, three miles from Pago Pago Harbor. Collected July 1920. Analyzed by D. M. Greenberg.
6. Gulf Stream, one mile from Loggerhead Key, Dry Tortugas. Collected July 1922. Analyzed by D. M. Greenberg.
7. Pacific Ocean, off Coast of California. Exact location lost. Analyzed by A. Gordon.
8. Pacific Ocean, off Coast of California. Exact location lost. Analyzed by A. Gordon.
9. Pacific Ocean, off Coast of California. Exact location lost. Analyzed by D. M. Greenberg.

PLAN OF ANALYTICAL WORK

The sea-water samples which, in most cases, consisted of two to five gallons each, were shipped to the laboratory after collection and kept in the dark at laboratory temperatures. So far as possible, the very latest modifications of the standard gravimetric, volumetric and colorimetric analytical methods were employed. Duplicate, and in some instances quadruplicate, samples were analyzed to check on the accuracy of the analysis. As indicated in the data furnished below, a much more complete analysis of each sample was made than is usually done in analysis of sea-water, and I believe that nearly all of the important constituents from the point of view of the physiologist are included. Some special determinations were made on additional samples which will be discussed below. The data obtained on the analyses are given in parts per million of the sea-water analyzed and also in milliequivalents, since the former may be quite misleading as regards the essential "function values" of the important ionic constituents. The data show, also, the balancing of the reaction values of the positive and negative ions. I have chosen to present the data in the form of the ionic constituents of sea-water rather than in some of the conventional forms employed commonly in inorganic quantitative analysis. Other features of the analytical work and its results are discussed below.

DISCUSSION OF THE DATA

The first disclosure in the data of Table 1, as the author studies them, is the negation which they give to the current assumption on the constancy and uniformity of the composition of sea-water. While it is doubtless true that there is a certain marked degree of relative uniformity in the composition of sea-water, our analyses show that the absolute differences between different samples are great enough to be of importance, not only for themselves but also because of their possible significance to living organisms inhabiting the ocean-waters in the surface 10 to 50 meters. Taking the next step into our appraisal of the data, we find that the variability in the composition of sea-water is even more marked as regards specific constituents than it appears to be in general. We may now discuss these general observations in greater detail.

TABLE 1—*Composition of sea-water samples*

Sample No.	1		2		3		4		5		6		7		8		9	
	Parts per million	Reaction values	Parts per million	Reaction values	Parts per million	Reaction values	Parts per million	Reaction values	Parts per million	Reaction values	Parts per million	Reaction values	Parts per million	Reaction values	Parts per million	Reaction values	Parts per million	Reaction values
pH	8.5		8.6		8.6						7.7							
Sp. Gr.	1.0253		1.025		1.025		1.023		1.023		1.027		1.025		1.023		1.023	
Conductivity referred to 1/50 N. KCl			15.06		15.18													
Total solids	35891.00		32741.00		33521.00		37500.00		36800.00		37750.00		37170.00		36350.00		34965.00	
Non-volatile solids	34018.00		27688.00		27902.00		34550.00		34950.00		34580.00		32500.00		32540.00		31490.00	
Volatile solids	1873.00		5053.00		5619.00		2950.00		1850.00		3170.00		4670.00		3810.00		3475.00	
SiO_2	11.10	0.18	6.00	0.10	6.00	0.10	6.00	0.10	4.00	0.07	9.80	0.16	7.50	0.125	9.00	0.150	1.95	0.030
Fe	0.12	0.006	0.14	0.008	0.13	0.007	5.50	0.30	3.00	0.16	1.50	0.08	0.30	0.016	0.30	0.016	0.40	0.020
Al			0.25	0.028	0.20	0.022	5.20	0.58	2.00	0.22	2.40	0.27	0.80	0.089	0.80	0.089	0.40	0.044
Ca	427.00	21.33	410.00	20.500	414.00	20.70	640.00	32.00	460.00	23.00	435.00	21.73	398.00	19.900	393.00	19.650	434.00	21.700
Mg	1313.00	108.00	1141.00	94.00	1156.00	95.20	1160.00	95.60	980.00	80.70	1325.00	109.00	1264.00	104.000	1237.00	101.700	1231.00	101.300
K	404.00	10.33	343.00	8.77	344.00	8.80	1010.00	25.82	945.00	24.20	435.00	11.12	381.00	9.740	381.00	9.740	412.00	10.540
Na	11050.00	481.00	9089.00	395.50	9152.00	398.00	10880.00	473.30	10475.00	456.00	11040.00	480.50	10850.00	472.000	10845.00	472.000	10185.00	443.000
SO_4	2649.00	55.18	2364.00	49.22	2418.00	50.30	2600.00	54.17	2700.00	56.22	2710.00	56.42	2545.00	53.000	2507.00	52.200	2478.00	51.600
PO_4	4.80	0.10	1.20	0.025	0.93		2.00	0.042	1.30	0.027	1.00	0.021	0.20		0.15		0.20	
CO_3	11.70	0.36											0.00		0.00		13.05	0.435
HCO_3	137.00	2.25					125.00	2.05	160.00	2.62	150.00	2.46	140.00	2.300	140.00	2.30		
Cl	19690.00	555.70	16869.00	476.00	17179.00	484.60	19350.00	545.80	19600.00	553.00	19960.00	563.00	18755.00	529.900	18580.00	524.00	18295.00	516.000
Total N	2.20						3.60		3.15		.35						0.00	
NaCl	28060.00		21792.00		21959.00		27650.00		26625.00									
KCl	768.00		1307.00		1310.00		1925.00		1800.00									
NaCl + KCl	28828.00		24569.00		24569.00													
Sum of cations	620.7		518.8		522.7		627.6		584.3		622.7		605.7		603.20		576.60	
Sum of anions	613.8		525.2		535.0		602.2		611.9		622.06		585.3		578.60		568.10	

The total solids are distinctly variable in the several samples analyzed, and this variability seems to be independent of the region from which the samples come. In regard to the volatile fraction of the total solids, the variability is much greater than for the total solids, indicating a marked divergence in the content of organic matter, carbonates and water of crystallization in the different samples. Here, again, we can find no correlation between the region from which the sea-water samples are taken and their analysis. For obvious reasons, the data for non-volatile solids require no special comment.

The data for SiO_2, Fe and PO_4 can not be regarded as accurate in all cases at least. The inadequacy of the method for determining SiO_2 leads me to regard all the figures for that constituent to be too high. Similarly for Fe, the figures for samples 4, 5 and 6 seem to be too high. The other figures for Fe may possibly be correct, since a more accurate method of determination was employed in those cases, but I should not stress their dependability too much. The figures for PO_4 in the samples seem to me to be too high. In the case of samples 7, 8 and 9, the most improved modification of the colorimetric method in use by Atkins in work above cited was employed and the data may, perhaps, be correct. In the other cases, the method was not sufficiently guarded and the results are not regarded as dependable.

In the case of Al, the alizarine method was employed in the analysis and may be regarded as dependable. For that reason, I should regard the data for all the samples except 4, 5 and 6, as nearly correct, but the data for the three exceptions as being too high.

Having disposed of the constituents of sea-water included in our analyses, which occur in very small quantities and for which the methods of analyses leave more or less to be desired, we may now study the data for the balance of the constituents on which the methods of analysis are good and which occur in relatively large quantities in sea-water. Starting with Ca, we are struck by the lack of constancy in the amount of that constituent in different samples of sea-water. The oft-repeated, but apparently unexamined assertion that sea-water is saturated with respect to $CaCO_3$, would seem to require some better support than our data furnish. To be sure, sea-water might contain a constant amount of $CaCO_3$ and $CaHCO_3$ in solution and still be variable respecting its content of other calcium compounds, but the data in Table 1 would not seem to render that likely. However that may be, it is quite certain from the foregoing data that the amount of calcium in sea-water is not constant. This is further emphasized by the figures for the reaction values for calcium in the several samples which serve not only to magnify the differences under discussion, but to emphasize the real differences existing in the composition of the different samples, especially as viewed by those who would apply these data to biological study. There is still no evidence that the region from which

the sample was obtained has any correlative value with the data resulting from analysis, although the highest reaction values for calcium seem to characterize the samples from tropical waters. It should be noted that sample No. 4 represents reef water from a small bay, and its notably higher Ca content is accounted for thereby. This argues also against the idea of saturation of sea-water with respect to $CaCO_3$.

What was said above is even more markedly true for Mg, K and Na than for Ca, and I would direct attention again to the difference shown by the figures in parts per million and that of the corresponding reaction values. In general, sea-waters in the tropics appear at least to have a higher content in Mg, K and Na than that in the regions of the temperate zone.

The SO_4 component seems to show the greatest uniformity of any of the constituents of sea-water, but it is clear from our data that it is far from being constant in amount in all the samples. Much the same holds true for the Cl component, but in both of these cases we note again the tendency for higher concentrations to occur in the samples from the tropics than in those from the temperate zone.

The other data given in the tables require no special comment, but the reader's attention is directed to the balancing of the cations and anions as a result of the reaction values furnished with the analyses.

The findings of physiologists in the last two decades or more on the importance of antagonism between ions and physiological balance, and especially in such concentrated media as sea-water, render the revelations in the foregoing analytical data of significance. Seeing as we do now, that the several important ions do not occur in sea-water everywhere in constant amounts, the suitability of the medium for different kinds of organisms would not be a constant, and the functional activity of different species of algæ, for example, in a given sea-water medium might be quite different. In another paper,[1] I have shown that the composition of *Lithothamnium* specimens from different oceanic areas may differ radically one from another. This is rendered more readily comprehensible in the light of the analyses submitted in this paper.

STUDIES SUPPLEMENTAL TO THE FOREGOING

Four additional samples of sea-water taken within a range of approximately 5° latitude and 4° longitude, off the California coast, were analyzed in a much more limited way than those discussed above. Because of their interest for themselves and their bearings on the foregoing data, these additional data are given as follows:

1. Off Santa Cruz Island, September 1923. Lat. 34° 5′ 45″ N., Long. 119° 55′ W. Conductivity times that of 1/50 N. KCl=16.32; Al content=0.17 p.p.m.; pH 8.64.

[1] *Chemical Composition of Lithothamnium from Various Sources.* Carnegie Inst. Wash. Pub. No. 340, 193-200. 1924.

2. At Cortes Bank buoy, September 1923. Lat. 32° 24′ 52″ N., Long. 119° 7′ 40″ W. Conductivity times that of 1/50 N. KCl.=16.32; Al content=0.17 p.p.m.; pH 8.47.
3. Lat. 37° 47′ 30″ N., Long. 123° 10′ W., October 1923. Conductivity times that of 1/50 N. KCl=16.26; Al content 0.25 p.p.m.; pH 8.64.
4. Off Santa Cruz. Lat. 36° 44′ N., Long. 122° 20′ W., October 1923. Conductivity times that of 1/50 N. KCl=16.39; Al content 0.17 p.p.m.; pH 8.57.

The data for pH of the foregoing samples are, of course, of little value, except to show the generally high pH of sea-water, since that datum will vary diurnally and in other ways to so large an extent. The figures for Al content are, however, quite significant and supplement and support the statements made in reference to the other analyses for aluminum. That the northernmost of the four samples under consideration contains more aluminum than the rest seems to me to be significant. Most interesting of all the data, however, are those for the conductivities as referred to 1/50 N. solutions of KCl. When these data are compared with the two figures for conductivities given in the foregoing tables, it is apparent that the concentrations of electrolytes may be markedly different as a whole in different samples of sea-water. This is true, despite the fact that data for specific gravity given for all the samples in the tables do not disclose the characteristic differences revealed by the conductivity determinations. It is to be much regretted that determinations of the conductivities of all the samples were not made.

CHANGES IN HYDROXYL ION CONCENTRATION AS AFFECTING THE COMPOSITION OF SEA-WATER

It is, of course, well known that some constituents of ordinary solutions are much influenced in their solubility by changes in the equilibrium concentrations of hydrogen and hydroxyl ions. It may not be so fully appreciated, however, to how great an extent this has a bearing in a practical way on the composition of sea-water and especially on its suitability as a medium for organisms under the rapidly changing concentrations of hydroxyl ion which characterize it in nature. Studies by a number of investigators in recent years have shown that the diurnal range in the hydroxyl ion concentration of both fresh waters and sea-waters supporting algal flora is very great. Thus changes of pH from 7.2 to 9.4 or similar ones have been noted in both kinds of media in the course of 12 hours or less. With these facts in mind, it occurred to the writer that it would be of interest to learn how large a part of some important constituents of sea-water are taken out of solution by raising the pH thereof from approximately 8.0 (commonly found for sea-water samples with little or no algal growth) to something more nearly approaching a value of 9. Accordingly, two samples of sea-water of 25 c.c. each were treated, respectively, with 2 c.c. N/10 KOH and 6 c.c.

N/10 NH_4OH. The pH was raised to 8.87 in the first sample and to 8.86 in the second by the addition of the alkali. Table 2 gives the results of an analysis of the precipitate which formed in the sample in each case and indicates the percentage of the total amount of each of the constituents determined in the sea-water sample which was rendered insoluble by the addition of the alkali.

TABLE 2—*Analysis of Precipitates*

	Sample 1		Sample 2	
	p.p.m.	Per cent of amount in 25 c.c. of sea-water	p.p.m.	Per cent of amount in 25 c.c. of sea-water
Fe_2O_3	2.4	8.00	2.4	8.00
P_2O_5	1.2	61.60	1.2	61.60
Al_2O_3	6.0	30.00	9.6	20.99
Ca	110.0	24.00	100.0	21.80
Mg	90.0	9.20	85.0	8.70

The data in the foregoing table render it clear that a large part of the ions important to algæ is removed from solution in sea-water by a rise in pH of that medium, which is well within the range of daily rise in pH of sea-water carrying an active algal flora under the proper conditions of light and temperature. That such a phenomenon occurs coincidentally with the marked lessening in absorption of the ions by the algæ is neither certain nor significant to the discussion in this paper. If, as appears likely to the author, the increase in pH comes at a time when absorption of ions by algæ is still very active, our facts and their discussion may be of very great significance to the whole problem of crops of algæ in the sea and incidentally also in fresh waters. It must be remembered, moreover, that the change under consideration from a lower to a higher pH takes place gradually in nature and some part of the phenomenon of precipitation of salts will be occurring all through a given day, hence being of significance throughout that period and not merely at the end of the day. The analyses given in Table 2 constitute only a beginning in such kind of study and it is highly desirable in my view to obtain more such analyses and to make them cover the ions of sea-water more completely.

I am indebted to Captain J. C. Rhodes of the U. S. Lighthouse Service, for the sea-water samples from the temperate zone of the Pacific. It is a great pleasure to express my heartfelt thanks to him here.